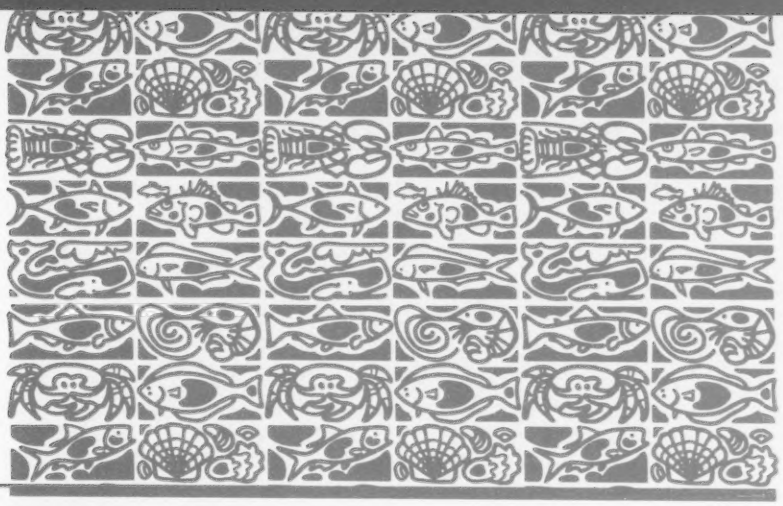

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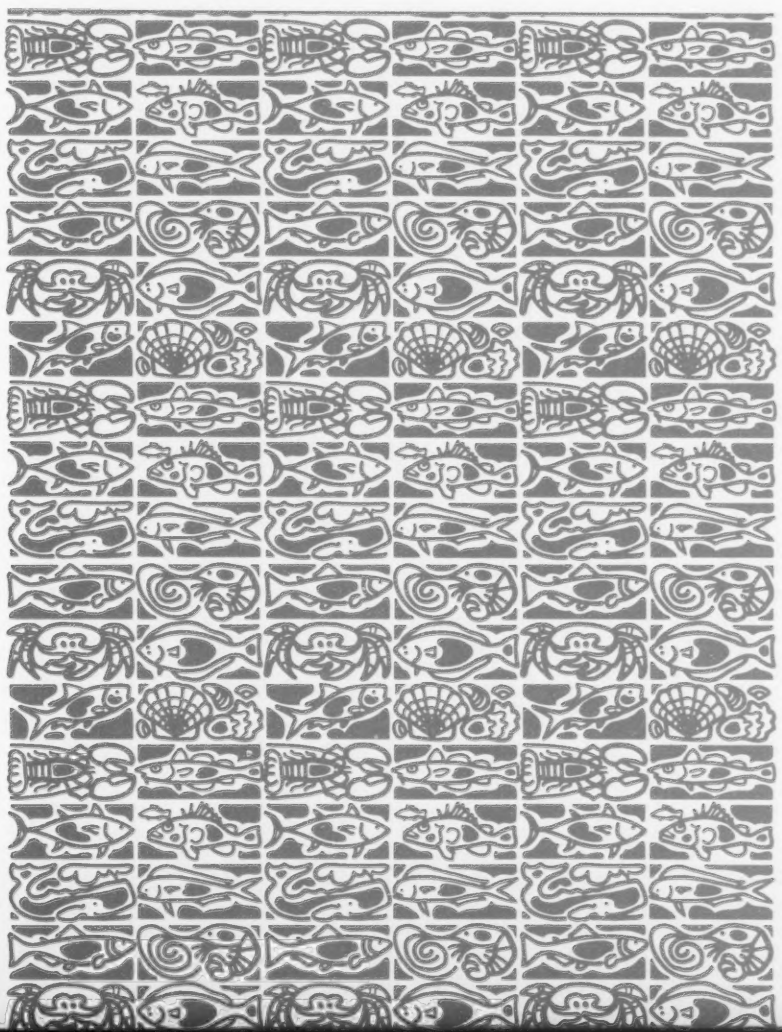

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Volume 99
Number 3
July 2001

Fishery Bulletin

Contents

Articles

389–398 **Andrews, Allen H., Erica J. Burton, Kenneth H. Coale, Gregor M Cailliet, and Roy E. Crabtree**
Radiometric age validation of Atlantic tarpon, *Megalops atlanticus*

399–409 **Colura, Robert L., and Britt W. Bumguardner**
Effect of the salt-box catch-bycatch separation procedure, as used by the Texas shrimp industry, on short-term survival of bycatch

410–419 **Gillanders, Bronwyn M.**
Trace metals in four structures of fish and their use for estimates of stock structure

420–431 **Hanrahan, Brian, and Francis Juanes**
Estimating the number of fish in Atlantic bluefin tuna (*Thunnus thynnus thynnus*) schools using models derived from captive school observations

432–442 **Lucena, Flávia M., and Carl M. O'Brien**
Effects of gear selectivity and different calculation methods on estimating growth parameters of bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae), from southern Brazil

443–458 **McBride, Richard S., Timothy C. MacDonald, Richard E. Matheson Jr., David A. Rydene, and Peter B. Hood**
Nursery habitats for ladyfish, *Elops saurus*, along salinity gradients in two Florida estuaries

459–464 **McFarlane, Gordon A., and Jacquelynne R. King**
The validity of the fin-ray method of age determination for lingcod (*Ophiodon elongatus*)

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465–474 **Rabe, Jessica, and Joseph A. Brown**
The behavior, growth, and survival of witch flounder (*Glyptocephalus cynoglossus*) larvae in relation to prey availability: adaptations to an extended larval period

475–481 **Schubart, Christoph D., Jesús E. Conde, Carlos Carmona-Suárez, Rafael Robles, and Darryl L. Felder**
Lack of divergence between 16S mtDNA sequences of the swimming crabs *Callinectes bocourti* and *C. maracaiboensis* (Brachyura: Portunidae) from Venezuela

482–501 **Sturdevant, Molly V., Audra L. J. Brase, and Leland B. Hulbert**
Feeding habits, prey fields, and potential competition of young-of-the-year walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, 1994–1995

502–509 **Sun, Chi-Lu, Chien-Lung Huang, and Su-Zan Yeh**
Age and growth of the bigeye tuna, *Thunnus obesus*, in the western Pacific Ocean

Note

510–515 **Takahashi, Kazutaka, and Kouichi Kawaguchi**
Nocturnal occurrence of the swimming crab *Ovalipes punctatus* in the swash zone of a sandy beach in northeastern Japan

516 *Errata*

517 *Subscription form*

Radiometric age validation of Atlantic tarpon, *Megalops atlanticus*

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Abstract—An improved radiometric aging technique was used to examine annulus-derived age estimates from otoliths of the Atlantic tarpon, *Megalops atlanticus*. Whole otoliths from juvenile fish and otolith cores, representing the first 2 years of growth, from adult fish were used to determine ^{210}Pb and ^{226}Ra activity; six age groups consisting of pooled otoliths and nine individual otolith cores were aged. This unprecedented use of individual otolith cores to determine age was possible because of improvements made to the ^{226}Ra determination technique. The disequilibria of ^{210}Pb:^{226}Ra for these samples were used to determine radiometric age. Annulus-derived age estimates did not agree closely with radiometric age determinations. In most cases, the precision (CV≤12%) among the otolith readings could not explain the differences. The greatest radiometric age was 78.0 yr for a 2045-mm-FL female, where the radiometric error encompassed the annulus-derived age estimate of 55 yr by about 4 yr. The greatest radiometric age for males was 41.0 yr for a 1588-mm-FL tarpon, where the radiometric error encompassed the annulus-derived age estimate of 32 yr by 1 yr. Radiometric age determinations in this study indicated that the interpretation of growth zones in Atlantic tarpon otoliths can be difficult, and in some cases may be inaccurate. This study provides conclusive evidence that the longevity of the Atlantic tarpon is greater than 30 years for males and greater than 50 years for females.

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The Atlantic tarpon (*Megalops atlanticus*) is believed to be a long-lived fish. The strongest evidence is from a captive female Atlantic tarpon that lived to at least 63 years when it died in 1998 at the John G. Shedd Aquarium in Chicago, Illinois.[1] Life in the aquarium, however, is difficult to compare with life in the natural environment. A similar longevity of 55 years was estimated from growth zones in sagittal otoliths of a wild female tarpon (Crabtree et al., 1995). The annual periodicity of the growth zones used to estimate age was validated by using oxytetracycline up to an age of 9 yr in captive fish (Crabtree et al., 1995), but extrapolation of these results to older, wild fish, however, is fallible. In addition, wild Atlantic tarpon are highly migratory and inhabit waters that vary considerably in salinity (0 to 43 ppt) and temperature (17° to 37°C; Zale and Merrifield, 1989; Nichols[2]). It is uncertain what affect these changing conditions have on the formation of growth zones in the otoliths, but periods of stress have been shown to halt otolith growth (Campana, 1983). Furthermore, growth slows with age and growth zones become increasingly compressed, rendering otolith growth zones difficult to interpret. To circumvent the potential problems of otolith interpretation and to independently determine age, an application of the radiometric aging technique by using the ^{210}Pb:^{226}Ra disequilibria in sagittal otolith cores (Campana et al., 1990) of Atlantic tarpon was performed, and the results are discussed in the context of existing estimated growth parameters.

Materials and methods

Pooled and individual otolith cores from Atlantic tarpon were analyzed for ^{210}Pb and ^{226}Ra to determine age. Annulus-derived age estimates were based on six independent otolith readings determined by Crabtree et al. (1995) and resulted in a coefficient of variation of less than 12%. For radiometric age determination, otoliths were pooled into six groups based on annulus-derived age estimates, sex, and collection date. In addition, nine individual otoliths were analyzed. The age groups were selected to cover the full range of annulus-derived age estimates. Selected age groups had a narrow age range and members of the group were collected within a 6-month period. Otoliths of adult age groups were cored to the first two years of growth and otoliths of the youngest age groups

[1] Pamper, K. 1999. Personal commun. John G. Shedd Aquarium, 1200 S. Lakeshore Dr., Chicago, IL 60605.

[2] Nichols, K. M. 1994. Age and growth of juvenile tarpon, *Megalops atlanticus*, from Costa Rica, South Carolina and Venezuela. Senior thesis, Univ. South Carolina, Columbia, SC 29208, 52 p.

were analyzed whole because they were similar in size to cores from adult fish. Because 2-yr cores were large and the activity of ^{226}Ra was relatively high (~10 to 100 times higher than usual; Andrews et al., 1999b), single otoliths were analyzed with the radiometric aging technique.

Coring the adult otoliths required establishing a target size and weight that closely approximated that of an otolith from a 2-year-old fish. Dimensions and weights of otoliths from four juvenile fish aged 2 years were recorded and averaged; the resultant dimensions were approximately 12 mm long by 6 mm high by 1 mm thick and a weight of 0.1 g. Each otolith from each adult age group was sculpted into this shape and weight by being hand-ground on a Buehler Ecomet® III lapping wheel. All samples were cleaned of any adhering contamination by following specific procedures described elsewhere (Andrews et al., 1999b). These clean samples were placed in acid-cleaned 100-mL Teflon® PFA Griffin beakers and dried at 85°C for 48 h.

A detailed protocol describing sample preparation, chromatographic separation of ^{226}Ra from barium and calcium, and analysis of ^{226}Ra using thermal ionization mass spectrometry (TIMS) is described elsewhere (Andrews et al., 1999b). Only an overview of the ^{226}Ra procedures is given here with details on the determination of ^{210}Pb activity. Because the levels of ^{226}Ra and ^{210}Pb typically found in otoliths were extremely low (from femtograms [10^{-15} g] for ^{226}Ra and attograms [10^{-18} g] for ^{210}Pb) and because of the great potential for contamination from calcium, barium, and lead, trace-metal clean procedures and equipment were used throughout sample preparation, separation, and analysis. All acids used were double distilled (GFS Chemicals®) and dilutions were made with Millipore® filtered Milli-Q water (18 MΩ/cm).

To determine ^{226}Ra activity with thermal ionization mass spectrometry (TIMS), the sample must be clean of naturally occurring organics (such as otolin). Organic residues elevate background counts in the ^{226}Ra region and increase the analytical uncertainty during TIMS analysis. Dried and weighed samples were dissolved in beakers on hot plates at 90°C by adding 8N HNO_3 in 1–2 mL aliquots. Alternation between 8N HNO_3 and 6N HCl, with an aqua regia transition, several times resulted in complete sample dissolution. The dried sample, after dissolution, formed a yellowish foam. To further reduce any remaining organics, and to put the residue into the chloride form required for the ^{210}Pb activity determination procedure, the samples were redissolved in 1 mL 6N HCl and taken to dryness five times at ~90°C. A whitish residue indicated that a sufficient amount of the organics had been removed. These samples were used to determine ^{210}Pb activity prior to TIMS analysis.

Determination of ^{210}Pb Activity

To determine ^{210}Pb activity in the otolith samples, the alpha-decay of ^{210}Po was used as a daughter proxy for ^{210}Pb. To ensure that activity of ^{210}Po was due solely to ingrowth from ^{210}Pb, the time elapsed from capture to ^{210}Pb determination was greater than 2 yr. Samples prepared for ^{210}Po analysis were spiked with ^{208}Po, a yield tracer. The amount of ^{208}Po added was estimated on the basis of observed ^{226}Ra levels in otoliths of juvenile tarpon. This amount was adjusted to five times the expected ^{210}Po activity in the otolith sample to reduce error in the ^{210}Pb activity determination. The spiked samples were redissolved in approximately 50 mL of 0.5N HCl on a hot plate at 90°C covered with a watch glass. The ^{210}Po and ^{208}Po-tracer were autodeposited for 4 hours onto a silver planchet (Flynn, 1968). The activities of these isotopes were determined by using alpha-spectrometry on the plated samples. Quantification of the ^{210}Po was made by subtracting a detector blank and reagent counts from each peak region-of-interest, by multiplying the ^{210}Po:^{208}Po count ratio by the known ^{208}Po activity, and by correcting for decay back to the time of plating. To attain sufficient counts, samples were counted for 21–23 d. The solution remaining after polonium plating was dried and saved for ^{226}Ra analyses.

Determination of ^{226}Ra Activity

To prepare the samples for ^{226}Ra activity determination with TIMS, each sample was spiked with ^{228}Ra, a yield tracer, and a newly developed ion-exchange separation technique was used to isolate radium from calcium and barium (Andrews et al., 1999b). The final samples were processed by using TIMS and the measured ratios of ^{226}Ra:^{228}Ra were used to calculate ^{226}Ra activity. The analysis of unspiked otolith samples indicated that ^{228}Ra was not present in measurable quantities in otoliths of juvenile fish, and no adjustment was necessary for the measured ^{226}Ra:^{228}Ra ratio in spiked samples.

Radiometric age determination

To assess the feasibility of applying the radiometric aging technique to Atlantic tarpon, uptake of ^{210}Pb and ^{226}Ra was assessed in otoliths from juvenile fish. Because the age of juvenile Atlantic tarpon is better constrained than that of adults, age was determined by using ^{210}Pb:^{226}Ra disequilibria in whole otoliths from juvenile fish. For the juvenile otolith samples, the age determined would be higher than expected if a significant amount of exogenous ^{210}Pb was incorporated into the otolith.

Age was estimated from the measured ^{210}Pb and ^{226}Ra activities (Eqs. 1 and 2). Because the activities were measured from the same sample, the calculation was independent of sample mass. For adult samples, where estimated age was greater than that of the 2-year-old core, radiometric age was calculated as follows with an equation derived from Smith et al. (1991) to compensate for the ingrowth gradient of ^{210}Pb:^{226}Ra in the otolith core,

$$t_{age} = \frac{\ln\left(\dfrac{1-\dfrac{A^{210}Pb_{tc}}{A^{226}Ra_{TIMS}}}{(1-R_0)\left(\dfrac{1-e^{-\lambda T}}{\lambda T}\right)}\right)}{-\lambda} + T, \quad (1)$$

where t_{age} = the radiometric age at the time of capture;
$A^{210}Pb_{tc}$ = the ^{210}Pb activity corrected to time of capture;
$A^{226}Ra_{TIMS}$ = the ^{226}Ra activity measured with TIMS;
R_0 = the activity ratio of ^{210}Pb:^{226}Ra initially incorporated;
λ = the decay constant for ^{210}Pb (ln(2)/22.26 yr); and
T = the core age (2 yr).

The radiometric age calculation for the juvenile age-group was determined by iteration of an equation derived from Smith et al. (1991),

$$\frac{A^{210}Pb_{tc}}{A^{226}Ra_{TIMS}} = 1-(1-R_0)\left(\frac{1-e^{-\lambda t_{age}}}{\lambda t_{age}}\right), \quad (2)$$

where all equation components were as defined above. A radiometric age range, based on the analytical uncertainty, was calculated for each sample by applying the calculated error for ^{210}Pb and ^{226}Ra activity determinations to the measured ^{210}Pb:^{226}Ra. Calculated error included the standard sources of error (i.e. pipetting, spike, and calibration uncertainties), alpha-counting statistics for ^{210}Pb (Wang et al., 1975), and an analysis routine used to run ^{226}Ra samples on the thermal ionization mass spectrometer (Andrews et al., 1999b).

Age estimate accuracy

To compare annulus-derived age with radiometric age, a plot of the annulus-derived age estimate and measured ^{210}Pb:^{226}Ra activity ratio was compared graphically to the expected ^{210}Pb:^{226}Ra activity ratio from ingrowth. This comparison included a graphical compensation for the ^{210}Pb:^{226}Ra gradient in the core sample. This model assumed a linear mass-growth rate for the first two years of growth. Annulus-derived age range and the analytical uncertainty of the activity ratio were plotted with each data point. A direct comparison of annulus-derived age and radiometric age was made in a plot where a regression of the data was compared to a line of agreement or slope of one. A paired two-sample *t*-test was used to determine if a significant difference existed between the age estimates.

Von Bertalanffy growth functions were fitted to the radiometric ages with FISHPARM software (Saila et al., 1988) and plotted with the annulus-derived ages and growth functions from Crabtree et al. (1995) for a visual comparison. High and low radiometric age and the size range of each sample were plotted for each data point. No statistical comparison was made between the growth functions because of the low number of samples and wide confidence interval for each parameter.

Results

Six age groups and nine individual otolith cores were selected for radiometric analyses (Table 1). Of the age group samples, three male samples and three female samples were selected to span the estimated age range of each sex. Male age groups were 2–4 yr, 18–21 yr, and 31–36 yr. Female age groups were 3–4 yr, 15–24 yr, and 48–50 yr. For each age group the capture dates were within a 6-month period, except the 2–4 yr male age group where the period spanned 7 months. Individual otolith core samples ranged in annulus-derived age from 13 yr to 32 yr for males and 35 yr to 55 yr for females. The greatest annulus-derived ages were for females; the oldest female was estimated to be 55 yr and the oldest male was 36 yr. Fork length was lowest for the juvenile age groups and did not overlap with older age groups. There was some overlap between the middle and old age groups. Males were typically smaller than females; the largest male was 1620 mm FL and the largest female was 2045 mm FL. The number of otoliths used in each age group ranged from 4 to 11 with the fine-cleaned sample weight ranging from 0.3314 to 1.0718 g. The individual core samples ranged in weight from 0.0884 g to 0.1366 g. These samples are the lowest weights and the first individual otolith cores ever used for radiometric age determination.

Activities of ^{210}Pb and ^{226}Ra were determined and combined to form an activity ratio for each sample (Table 2). The ^{210}Pb activity spanned a wide range and increased from juveniles to adults by as much as 88 times. The lowest activities were for the juvenile samples (0.003 and 0.005 disintergrations per gram [dpm/g]) and the highest activity was 0.265 dpm/g (±6.0%) for the largest male (1620 mm FL). The activity of ^{226}Ra varied by an order of magnitude and ranged from 0.044 dpm/g (±1.53%) to 0.401 dpm/g (±1.02%). Calculated ^{210}Pb:^{226}Ra ranged, as predicted, between 0 and 1; the lowest activity ratios were for the juvenile samples and the highest were for large adults. All low and high ratios were within the limits of 0 to 1 (values >1 are mathematically undefined; Eqs. 1 and 2) except for the largest female (2045 mm FL), which had an upper limit that exceeded 1. Radiometric age of the juvenile samples was very close to the expected age (Table 3). Exogenous ^{210}Pb was, therefore, either not present or present in negligible quantities. This was inferred to be true for the core, or juvenile region, of adult Atlantic tarpon otoliths.

Comparison of annulus-derived ages and radiometric ages indicated there were differences in the aging results for each technique (Table 3). In most cases, the precision among the otolith readings could not explain the differences. In four cases, the different age estimates overlapped, but the extent of overlap was at the extreme of the age range. The lowest radiometric ages were one year for the samples from juvenile fish and the highest was 78.0 yr for the largest adult female.

A graphical comparison of the expected ^{210}Pb:^{226}Ra disequilibria from ingrowth with the measured ^{210}Pb:^{226}Ra indicated there was variation in the measured results above and below what was expected (Fig. 1). The low radiometric age for the juvenile age-groups indicated that uptake of exogenous ^{210}Pb (R_0; Campana et al., 1990) by juveniles was insignificant. Therefore, the best model for ingrowth of ^{210}Pb from ^{226}Ra in tarpon otoliths was for R_0 = 0.0.

Table 1

Detailed summary of data for pooled otolith age groups and single otoliths from *Megalops atlanticus*. Age range of age groups is based on annulus-derived age estimates. Capture dates and average fork length are listed for comparison. Age groups consisted of 4 to11 otolith cores and amounted to ~1 g. Single otolith cores weighed close to 0.1 g.

Age-group or single otolith age (yr)	Capture date or range	Fork length (mm ±SD)	Number of otoliths	Sample weight (g)
Male				
2–4[1]	7 Mar–16 Oct 1989	568 ±80	7	0.6786
13	22 Jun 1989	1499	1	0.1129
17	4 Jul 1989	1452	1	0.1124
18–21	1 May–25 Jul 1989	1442 ±78	10	1.0498
31–36	12 Jun–7 Jul 1990	1532 ±73	4	0.3480
32	6 Jun 1989	1588	1	0.1279
32	19 Jul 1992	1620	1	0.1220
Female				
3–4[1]	24 Jan–15 Jul 1989	583 ±59	9	0.8775
15–24[2]	6 Jun–12 Aug 1989	1641 ±92	11	1.0718
35	4 Jul 1989	1613	1	0.1002
36	12 May 1992	1780	1	0.1331
37	12 Jun 1993	1950	1	0.1167
44	2 Sep 1991	2040	1	0.1366
48–50	20 Apr–20 Jun 1989	1708 ±110	4	0.3314
55	4 Mar 1991	2045	1	0.0884

[1] Whole juvenile otoliths.

[2] Ten otoliths ranging in age from 21 to 24 yr; one 15-yr-old otolith was erroneously included in this age group.

Table 2

Radiometric results for each *Megalops atlanticus* age group and single otolith samples. Activities are expressed as disintegrations per minute per gram (dpm/g).

Age-group or single otolith age (yr)	Sample weight (g)	^{210}Pb (dpm/g) ± % error[1]	^{226}Ra (dpm/g) ± % error[2]	^{210}Pb:^{226}Ra activity ratio	^{210}Pb:^{226}Ra low	^{210}Pb:^{226}Ra high
2–4[3]	0.6786	0.005 ±6.7	0.258 ±1.03	0.017	0.016	0.019
13	0.1129	0.043 ±12.1	0.085 ±1.52	0.507	0.439	0.577
17	0.1124	0.035 ±13.6	0.061 ±1.61	0.568	0.483	0.656
18–21	1.0498	0.072 ±3.4	0.246 ±1.01	0.292	0.280	0.305
31–36	0.3480	0.094 ±5.3	0.181 ±1.25	0.520	0.486	0.555
32	0.1279	0.031 ±13.5	0.044 ±1.53	0.712	0.607	0.821
32	0.1220	0.265 ±6.0	0.401 ±1.02	0.663	0.617	0.710
Female						
3–4[3]	0.8775	0.003 ±5.7	0.217 ±1.06	0.015	0.014	0.016
15–24	1.0718	0.059 ±3.9	0.163 ±1.07	0.362	0.345	0.381
35	0.1002	0.119 ±8.2	0.299 ±1.35	0.397	0.360	0.435
36	0.1331	0.086 ±8.5	0.144 ±2.24	0.597	0.534	0.663
37	0.1167	0.126 ±8.0	0.243 ±1.18	0.518	0.471	0.567
44	0.1366	0.113 ±7.4	0.170 ±1.33	0.667	0.609	0.726
48–50	0.3314	0.148 ±4.5	0.266 ±1.19	0.555	0.524	0.587
55	0.0884	0.086 ±12.2	0.094 ±1.42	0.909	0.787	1.035

[1] Calculation based on standard deviation of ^{210}Pb activity; Wang et al., 1975.

[2] Calculation based on TIMS analysis routine (±1 SE).

[3] Whole juvenile otoliths.

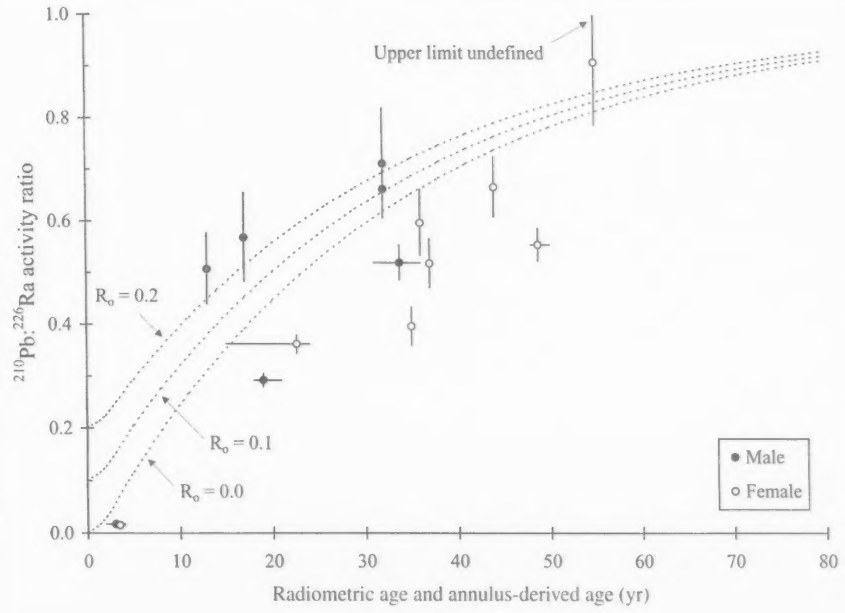

Figure 1

Expected ^{210}Pb:^{226}Ra ingrowth curves and observed ^{210}Pb:^{226}Ra activity ratios for male and female Atlantic tarpon (*Megalops atlanticus*) individuals and age groups. Expected ingrowth curves represent initial uptake ratios (R_0) of 0.0, 0.1, and 0.2. Expected ^{210}Pb:^{226}Ra ingrowth during early otolith growth (until core-age) is based on a linear mass-growth model. After core growth, the secular equilibrium model for ^{210}Pb:^{226}Ra resumes and continues to unity. Data points are annulus-derived ages (average age for age groups) plotted against measured ^{210}Pb:^{226}Ra activity ratios. Vertical bars represent analytical uncertainty of ^{210}Pb and ^{226}Ra measurements. Horizontal bars represent the range of annulus-derived ages for age groups. The upper limit of the ^{210}Pb:^{226}Ra ratio for the oldest female exceeded 1.0; therefore, high radiometric age is mathematically undefined (Eq. 2).

Table 3

Comparison of annulus-derived ages and radiometric ages for *Megalops atlanticus*. The mean age of each group is based on annulus-derived age estimates. The radiometric age range is based on low and high activity ratios from analytical uncertainty calculations. The radiometric age calculations are based on the measured ratio of ^{210}Pb:^{226}Ra.

Age-group or single otolith age (yr)	Mean annulus-derived age (yr)	Radiometric age range (yr)	Radiometric age (yr)	Age-group or single otolith age (yr)	Mean annulus-derived age (yr)	Radiometric age range (yr)	Radiometric age (yr)
Male				Female			
2–4	3.0	1.1–1.2	1.1	3–4	3.4	0.9–1.1	1.0
13	13	19.6–28.7	23.7	15–24	22.6	14.6–16.4	15.5
17	17	22.2–35.3	28.0	35	35	15.3–19.3	17.2
18–21	19.0	11.5–12.7	12.1	36	36	25.5–35.9	30.2
31–36	33.8	22.4– 27.0	24.6	37	37	21.5–27.8	24.5
32	32	31.0–56.2	41.0	44	44	31.2–42.6	36.3
32	32	31.8 – 40.8	35.9	48–50	48.8	24.8–29.4	27.0
				55	55	50.6–undefined[1]	78.0

[1] Upper radiometric age limit for the 78-yr female is undefined because the ^{210}Pb:^{226}Ra ratio exceeded 1.0.

Hence, radiometric age was determined on the basis of measured activity ratios and no adjustment for exogenous ^{210}Pb was necessary. When the radiometric and annulus reading (±12% CV) age ranges were considered graphically, most of the data points still did not agree with the expected ingrowth curve (R_0=0.0).

A direct comparison of annulus-derived age with radiometric age indicated that the ages were evenly distributed on either side of a line of agreement and that the slope of the regression (slope=0.915) was close to 1 (Fig. 2). Radiometric age was not statistically different from the annulus-derived age estimates (paired two-tailed t-test, df=14, t=0.4181, P=0.6822). The coefficient of determination was low (adjusted r^2=0.55; Kvålseth, 1985) and the power of the test to detect significant differences was low because of the variability of the data and the small sample size. The analytical uncertainty associated with radiometric age determination or the reading range of the annulus-derived age encompassed the line of agreement for only four samples. The sample with the closest agreement was a female (1780 mm FL) whose annulus-derived age was 36 years. The annulus-derived age estimates of the juvenile age groups were high by 1.9 and 2.4 years. For older age groups and individual cores, annulus-derived age was higher than radiometric age by as much as 21.8 years and lower than radiometric age by as much as 23 years. Otolith sections that had radiometric and annulus-derived age estimates that differed considerably were photographed to illustrate the difficulty in aging otoliths (Fig. 3).

Von Bertalanffy growth functions fitted to the radiometric ages of males and females (Figs. 4 and 5) were similar to annulus-derived growth functions determined by Crabtree et al. (1995). However, the low number of radiometric data points resulted in large confidence intervals (±95%) for growth model parameters. Radiometric-age growth parameters for males indicated an asymptotic length (L_∞) of 1550 ±83 mm FL and a growth coefficient (k) of 0.19 ±0.12 (Fig. 4). Radiometric-age growth parameters for females indicated an L_∞ of 2030 ± 227 mm FL and a growth coefficient (k) of 0.08 ±0.04 (Fig. 5).

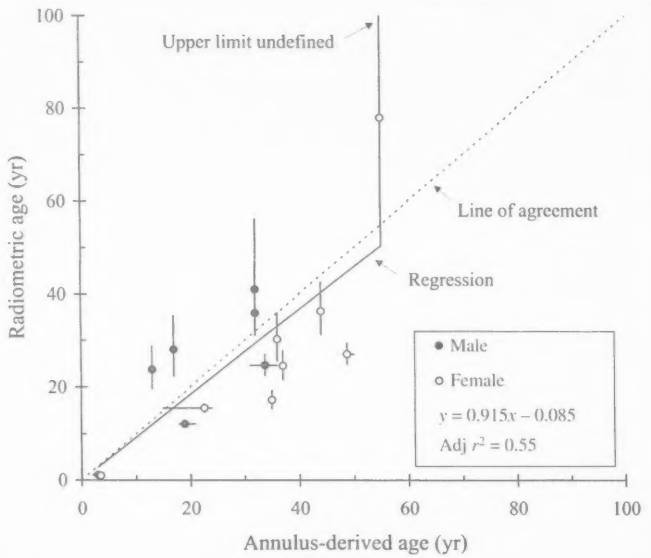

Figure 2

Comparison of male and female Atlantic tarpon (*Megalops atlanticus*) annulus-derived ages and radiometric ages. A linear regression and a line of agreement are plotted for comparison. Vertical bars represent low and high radiometric age estimates based on the analytical uncertainty of ^{210}Pb and ^{226}Ra measurements. Horizontal bars represent the range of annulus-derived ages for age groups. The upper limit of the radiometric age estimate for the oldest female is undefined because the analytical uncertainty of ^{210}Pb:^{226}Ra exceeds 1.0.

Discussion

In four cases, the radiometric age range encompassed the annulus-derived age estimate. The oldest female tarpon had an estimate (55 yr) that was lower than the radiometric age by 23 yr. The radiometric age range, however, encompassed the annulus-derived age by 4 years. This may indicate that the annulus-derived age estimate was correct in this case. The radiometric age validation, by itself, confirms the longevity of female Atlantic tarpon to at least 50.6 yr, but it may indicate that age can meet or exceed 78.0 yr (Table 3). Similarly, the longevity of male Atlantic tarpon was confirmed to at least 31.8 yr, but may exceed 41.0 yr. When the CV for annulus-derived age was included in the range of age estimates, some additional samples encompassed the radiometric age estimates, but most deviant ages could not be explained by this variation.

The wide dispersion of residuals and the low coefficient of determination indicated that there were differences between radiometric age and annulus-derived age that could be explained in the interpretation of otolith growth zones (Fig. 2). According to Crabtree et al. (1995), aging otoliths of the Atlantic tarpon was confusing and many were not readable. Although Crabtree et al. (1995) validated the annual periodicity of growth zone formation for 12 out of 18 young fish up to an age of 9 yr in captivity, the pattern of growth zone formation in older, wild fish may not be annual. Because the tarpon is a migratory fish that inhabits inshore and estuarine waters of varying salinity and temperature and spawns offshore (Zale and Merrifield, 1989; Crabtree et al., 1992), there is a high potential for irregular growth zone formation from the stresses of extreme changes in habitat (Pannella, 1980; Campana, 1983). Subannual growth zones may explain annulus-

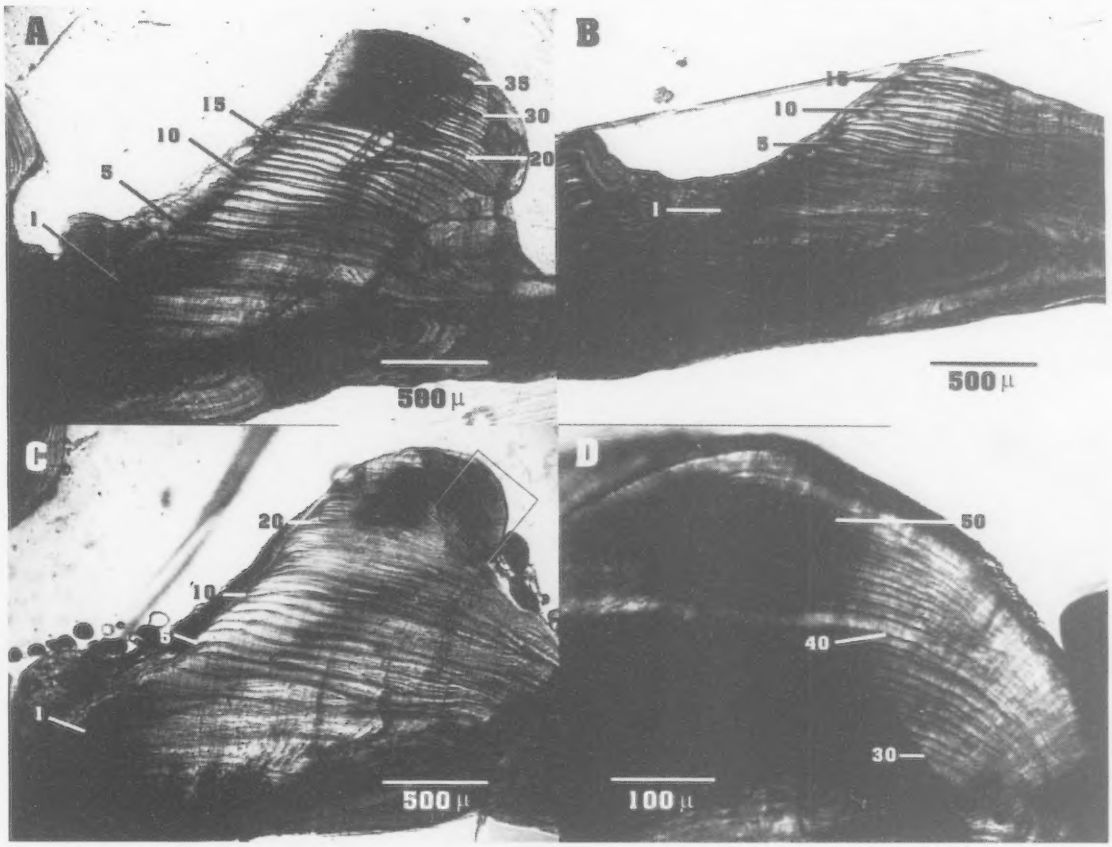

Figure 3

Transverse otolith sections from three Atlantic tarpon (*Megalops atlanticus*) illustrating the inherent difficulty in counting annuli. Annulus-derived ages were estimated by counting visible growth increments for each section shown (Crabtree et al. 1995). One sagitta was used for counting annuli and the other for radiometric aging. (**A**) An otolith section for which the number of growth increments clearly exceeds the radiometric age estimate. The annulus-derived age estimate was 37 yr and the radiometric age estimate was 24.5 yr. (**B**) An otolith section with fewer growth increments than the radiometric age estimate, but which could have had a higher annulus-derived age estimate if the reader had taken a more aggressive approach. The annulus-derived age estimate was 17 yr and the radiometric age estimate was 28.0 yr. (**C**) An otolith section with a well-developed sulcal region and numerous growth increments that rapidly become compressed toward the margin at the lower left. (**D**) Close up of the region in the same section (C) where growth increments are compressed and narrowly spaced. Note that growth increments in the region are partially obscured by what appears to be a proteinaceous inclusion. The annulus-derived age estimate was 55 yr and the radiometric age estimate was 78.0 yr.

derived age estimates that were higher than radiometric age. Poorly defined annual growth zones, or growth zones that become too small or compressed to be quantified, may explain annulus-derived ages that were lower than radiometric ages (Fig. 3). An additional consideration was that the age groups were composed of otoliths pooled together on the basis of annulus-derived age. This means that the radiometric age determined for these groups was the average age of the otoliths in the sample. Hence, a mixture of otoliths of differing ages would create an unaccountable error in the radiometric age of the age groups. This variability, however, was minimized by using otoliths of similar weight.

Von Bertalanffy growth functions fitted to the radiometric ages had growth parameters that were similar to the annulus-derived growth parameters (Crabtree et al., 1995). This determination, however, is subjective because 1) there were a low number of radiometric samples, 2) the confidence intervals were large, and 3) three out of seven male samples and three out of eight female sam-

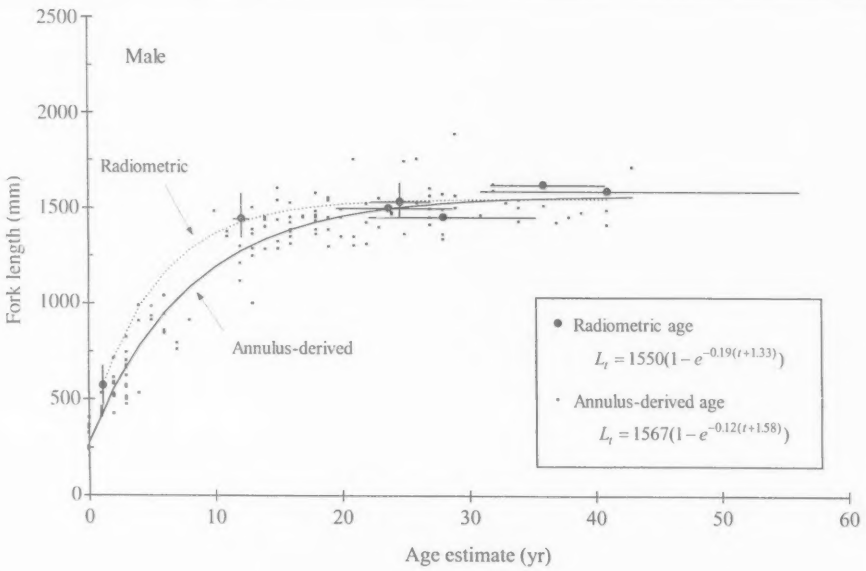

Figure 4

Von Bertalanffy growth curves for male Atlantic tarpon (*Megalops atlanticus*) with radiometric age (yr) and fork length (mm; average fork length for age groups) and with annulus-derived age (yr) and fork length. Vertical bars represent fork length range for age groups. Horizontal bars represent low and high radiometric age estimates.

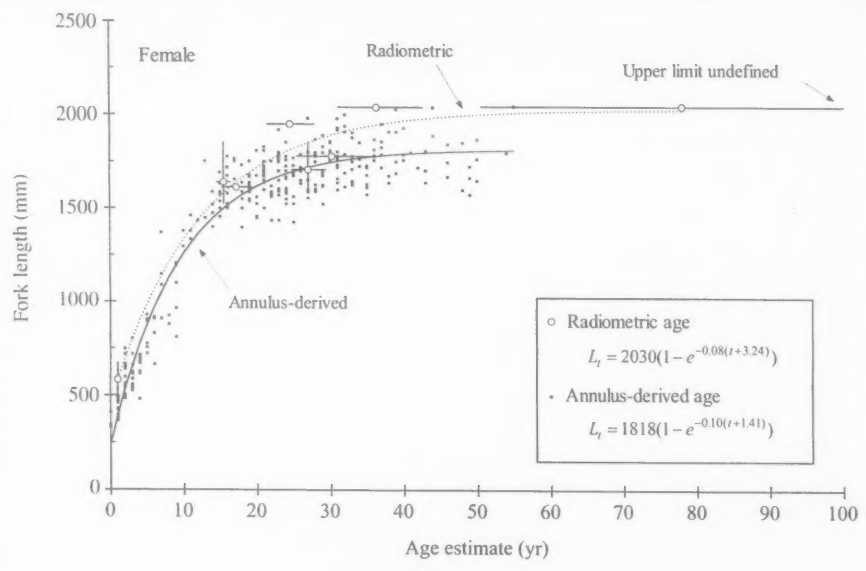

Figure 5

Von Bertalanffy growth curves for female Atlantic tarpon (*Megalops atlanticus*) with radiometric age (yr) and fork length (mm; average fork length for age groups) and with annulus-derived age (yr) and fork length. Vertical bars represent fork length range for age-groups. Horizontal bars represent low and high radiometric age estimates.

ples were an average age and average fork length for each age group. The growth coefficient for male radiometric ages (k=0.19 ±0.12) was similar to the annulus-derived estimate (k=0.12) according to the margin of error and was driven largely by one middle-age sample (Fig. 4). The growth coefficient for female radiometric ages (k=0.08 ±0.04) was also similar to the annulus-derived estimate (k=0.10). The male asymptotic length for radiometric ages (L_∞=1550 ±83 mm FL) encompassed the result from annulus counts (L_∞=1567 mm FL). For females, the asymptotic length was greater (L_∞=2030 ±227 mm FL) but still encompassed the result from annulus counts (L_∞=1818 mm FL). The closeness of the radiometric estimate of L_∞ to the maximum size was probably driven by the exceptionally large tarpon used in our study (Fig. 5).

The large size of the 2-year-old otolith core, coupled with the relatively high ^{226}Ra activity and high sensitivity of the TIMS technique, permitted age determination of smaller samples than previously possible (Kastelle et al., 1994; Fenton and Short, 1995). In a recent radiometric aging study of the blue grenadier (*Macruronus novaezelandiae*), six pooled otolith cores were needed to attain a sample weight of approximately 1 g with measurable ^{226}Ra activity (Fenton and Short, 1995). Although the ^{226}Ra activity for the samples in that study were a factor of 10 lower than the ^{226}Ra activities observed in our study, the technique used was less sensitive than TIMS and resulted in relatively high analytical uncertainties (12–21%). In a recent study of sablefish (*Anoplopoma fimbria*), where radium activities were similar to the activities observed in our study, the margin of error was relatively low (~4%), but otolith cores still needed to be pooled to attain approximately 1 g (Kastelle et al., 1994). The use of TIMS to determine ^{226}Ra in our study made it possible to age age-groups that were approximately one third of a gram and individual otolith cores that were less than 0.1 g with analytical uncertainties typically less than 2%. Because of this advance, the greatest contribution to the aging error was no longer from ^{226}Ra determination, but from the determination of ^{210}Pb by means of α-spectrometry (Andrews et al., 1999b). In our study, the determination of ^{210}Pb activity contributed from 77% to 90% of the aging error.

The radiometric aging technique is well supported as a valid aging tool in numerous fish aging studies (Bergstad, 1995; Burton et al., 1999). In our study, ^{226}Ra activities varied by approximately an order of magnitude, but the ^{210}Pb activities never exceeded the activity of ^{226}Ra. Therefore, it is highly unlikely that ^{210}Pb was not the result of ingrowth from ^{226}Ra incorporated during otolith formation. By performing a complete analysis on individual otoliths, we found that we could use radiometric age estimates with more confidence than estimates derived from pooled otolith samples. More recent successes with this technique (Andrews et al., 1999a) suggest that it is a reliable means to obtaining accurate age estimates. Radiometric age determinations in our study, therefore, indicate that the interpretation of growth zones in Atlantic tarpon otoliths can be difficult, and in some cases can be inaccurate. In addition, our study provides conclusive evidence that the longevity of the Atlantic tarpon is greater than 50 years.

Conclusions

Accurate age determination of heavily harvested fish species is critical to the formulation of responsible management strategies. The typical growth-zone validation techniques have limited applicability to long-lived fishes (Mace et al., 1990; McFarlane and Beamish 1995). Underestimated longevity and overfishing were factors that led to the decline of the Pacific ocean perch (*Sebastes alutus*) and the orange roughy (*Hoplostethus atlanticus*; Beamish, 1979; Smith et al., 1995). Because of the highly variable growth zone patterns (Fig. 3) and irregular life history of the Atlantic tarpon, conventional aging methods appear to be problematic for this species. The improved radiometric aging technique used in our study incorporated a growth-zone independent chronometer that, when judiciously applied, will enable accurate age determination of many threatened species. At a time when many fish species are suffering from fishing pressure and changing oceanographic conditions, new methods need to be applied to help ascertain appropriate management strategies. The radiometric aging technique, which has been successfully applied to the sablefish, whose longevity has been validated with other techniques (Kastelle et al., 1994, Beamish and McFarlane 2000), promises to be a valuable tool for aging species with unknown or difficult-to-interpret otolith growth patterns.

Acknowledgments

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Effect of the salt-box catch-bycatch separation procedure, as used by the Texas shrimp industry, on short-term survival of bycatch

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Abstract—Experiments were conducted to determine the effect of the salt-box catch-bycatch separation procedure, as used by the Texas shrimp industry, on short-term survival of bycatch. Bioassays were conducted on five economically important bycatch species: spotted seatrout (*Cynoscion nebulosus*); red drum (*Sciaenops ocellatus*); Atlantic croaker (*Micropogonias undulatus*); southern flounder (*Paralichthyes lethostigma*); and blue crab (*Callinectes sapidus*). Red drum were most affected by hypersalinity, requiring 17 minutes exposure to a 70‰ salt water solution to kill 50% of the test specimens within 48 hours. For samples collected from commercial boats and Texas Parks and Wildlife (TPW) trawl samples, we found that neither initial nor final percent survival was significantly different for bycatch removed with or without the aid of a salt-box. Bycatch mortality was high regardless of the method used to separate bycatch from the target catch. At the conclusion of catch separation, bycatch survival averaged 76% (±22%) for commercial samples and 48% (±40%) for TPW trawl samples separated with salt-boxes. Survival at the conclusion of catch separation without a salt-box averaged 56% (±35%) for commercial samples and 43% (±39%) for TPW trawl samples. Bycatch survival 21–27 h after catch separation averaged 13% (±6%) for commercial samples and 5% (±9%) for TPW trawl samples separated with salt-boxes and 34% (±29%) for commercial samples and 10% (±19%) in TPW trawl samples separated without a salt-box. Mortality rates (M) for bycatch separated with a salt-box averaged 0.08 (±0.03) for commercial samples and 0.10 (±0.04) for TPW trawl samples. For bycatch separated without the salt-box, M averaged 0.48 (±1.23) for commercial samples and 0.10 (±0.05) for TPW trawl samples. Results of an exploratory analysis with stepwise multiple regression suggested that final percent survival of bycatch was most affected by trawling time. The salt-box had little or no effect on bycatch survival; therefore, regulating the use of salt-boxes in shrimp trawling operations is not necessary.

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Shrimp (*Penaeus* spp.) are the most important commercial seafood product in Texas, accounting for more than 80% by weight and 93% of the value of all Texas commercial fisheries landings (Robinson et al., 1996). Texas shrimp landings from 1986 through 1995 were about 32,000–44,000 metric tons (t) annually. About 6400–8000 t of the annual catch during the 10-year period came from Texas bays. Texas bay shrimpers are reported to catch 2.4–6.8 kg of nontarget species (bycatch) for each kg of shrimp landed (Fuls and McEachron, 1998). Removal of the bycatch is time consuming and thus costly to fishermen. To reduce the time spent removing bycatch, Texas bay shrimpers frequently use salt-boxes to assist in rapid separation of bycatch from shrimp (Bumguardner and Colura, 1997). Salt-boxes are onboard tanks that hold a hypersaline solution of seawater and food-grade salt. The catch is placed in the solution, where most fish species float and shrimp sink. Floating bycatch is skimmed from the surface and discarded. Shrimp are then dipped from the tank and any remaining bycatch removed and discarded. Although use of salt-boxes is known to fisheries managers, the effect of its use on the survival of discarded organisms is unknown.

The objectives of our study were to assess the effects of the use of the salt-box on bycatch survival. This was accomplished by first conducting bioassays to determine the exposure time to hypersaline conditions that would affect survival of five economically important species known to occur as a part of the bycatch (Fuls and McEachron, 1998). The combined effects of trawling and exposure to hypersaline conditions in the salt-box were evaluated by comparing survival and mortality rates of bycatch (all species) and three major components of the bycatch (Atlantic croaker, *Micropogonias undulatus*, sand seatrout, *Cynoscion arenarius*, and spot, *Leiostomus xanthurus*) separated with and without the aid of a salt-box. This comparison was made for a series of samples collected from Texas bay shrimp fishermen and a series of samples collected during control experiments conducted by Texas Parks and Wildlife (TPW) personnel using experimental trawls. The TPW control experiments were conducted to remove some of the variation caused by the differing fishing methods used by each bay shrimper (i.e. different lengths of time the trawl was fished).

Materials and methods

Bioassays

Bioassays were conducted on spotted seatrout (*Cynoscion nebulosus*), red drum (*Sciaenops ocellatus*), Atlantic croaker, southern flounder (*Paralichthys lethostigma*), and blue crab (*Callinectes sapidus*). The species were selected because of their importance to Texas' recreational or commercial fisheries, or both (Weixelman et al., 1992; Robinson et al., 1996) and because they are known to occur as a portion of the bycatch (Fuls and McEachron, 1998). Red drums and blue crabs were col-

Table 1

Exposure times, salinity, temperature, and number of individuals per replicate used in high-salinity exposure bioassays. Three replicates were used at each exposure time.

Species	Exposure times (min)	Salinity (‰)	Temperature (°C)	Bay water salinity (‰)	Fish/ replicate
Red drum	4, 8, 16, 32, 64, 128	70	24	19	10
Atlantic croaker	4, 8, 16, 32, 64, 128	70	23	16	5
Spotted seatrout	4, 8, 16, 32, 64[1]	69	26	24	5
Southern flounder	4, 8, 16, 32, 64, 128	71	27	18	5
Blue crab	4, 8, 16, 32, 64, 128	70	26	24	5

[1] Specimens did not survive to 128 min of exposure.

lected from TPW hatchery ponds; Atlantic croaker and southern flounder were collected from Matagorda Bay with a trawl; spotted seatrout were collected form East Matagorda Bay with a bag seine. Fish were held 7–10 days in 1850-L tanks at ambient bay salinities and temperatures and fed frozen brine shrimp or chopped shrimp (or both) three times daily. Blue crabs were placed in holding tanks for one day prior to bioassay initiation. Juvenile blue crabs molt at frequent intervals and because of their aggressive nature, any soft crabs in a common tank are almost immediately cannibalized. Therefore, blue crab trials were held before substantial mortality occurred in holding tanks and without an initial acclimation period.

Bioassay trials were conducted at salinities of 70‰ (±1‰) and temperatures from 23 to 27°C. A salinity of 70‰ was selected on the basis of salinities observed in salt-boxes on commercial shrimping vessels (Bumguardner and Colura, 1997). Trials were conducted over a geometric sequence of times up to 128 minutes with three replicates for each time (Table 1). Either 5 or 10 individuals were exposed for each replicate, depending on size and availability. Test animals were placed in 4-L all-glass aquaria that had been washed with acid and alcohol and filled with about 3-L of 70‰ salt water. Air was continuously bubbled through the water from a 1-mL disposable pipette attached to an air blower. High-salinity water was obtained by adding salt (Mortons Ship n Shore®) purchased from a fishing supply store to water taken from Matagorda Bay. Salinity levels of bay water for each trial are presented in Table 1. Animals were removed from test aquaria at the end of the specified exposure time, high-salinity water was discarded, the aquarium was refilled with bay water, and animals were replaced in the aquaria. So that control animals were treated similarly, they were placed in the aquaria containing bay water and held for 60 minutes, after which these animals were removed, the bay water replaced with new bay water, and the control animals returned to the aquaria. The number of live individuals was recorded for each replicate immediately after high-salinity exposure, 24 hours after exposure, and 48 hours after exposure. For each species, probit analysis was conducted on untransformed data to determine the time at which 50% of the test animals exposed to the test salinity died (LE_{50}) (Sokal and Rohlf, 1981). A no-observed-effect exposure (NOEE) (Rand and Petrocelli, 1985) for each species was calculated by using a multiple goodness-of-fit comparison of log-likelihood ratio G values (Sokal and Rohlf,1981).

Effect of salt-box use on bycatch survival: samples from the bay shrimp industry

Thirty samples of bycatch were collected from May through October 1995 from bay shrimpers working in Matagorda, San Antonio, and Aransas bays. Fifteen samples were separated with a salt-box (SB) and the remaining 15 samples were separated without the aid of a salt-box (NSB). For each sample the following data were collected:

1 Length of time that trawl was fished;
2 Time that catch was removed from water;
3 Time that separation procedure began;
4 Time that bycatch was returned to water;
5 Bay temperature;
6 Bay salinity; and
7 Salinity in salt-box.

Total catch weight was not measured because bay shrimpers do not weigh individual catches and we did not want to change their shrimping practices more than necessary to collect the samples. To collect bycatch samples, an individual boarded the boat and waited until the bycatch was discarded. A 1–2 kg sample was randomly removed from the bycatch with a dip net or plastic scoop and placed into a 60-cm diameter × 60-cm deep holding pen. Pens were constructed from a bag of 15-mm stretch-mesh knotless nylon net secured to a life ring. The opening in the life ring served as the opening of the pen. After the sample was placed in the pen, the opening was covered with 6-mm stretch-mesh netting. The pen was then placed overboard where TPW personnel in another boat recovered it. The samples, when brought aboard the TPW boat, were immediately placed in separate 89-L transport tanks (ice chests fitted with plastic tubing and commercial aquarium air stones, through which compressed oxygen was supplied to the tanks). Dead organisms were removed from samples immediately after placement in the transport tanks.

All moribund specimens were classified as dead. Samples were transported to the Perry R. Bass Marine Fisheries Research Station (PRB) located near Palacios, Texas, where they were transferred to separate 60-cm diameter × 60-cm deep holding pens placed in an aerated 1500-L holding tank. Water in the holding tank was maintained within 2°C and 2‰ salinity of the bay temperature and salinity from which the samples were taken. Samples were checked at least hourly during transport to PRB. After having been placed in the holding tank, organisms were checked at least every three hours, except from 2300 to 0700 hours, when they were checked once. Each time samples were checked, beginning when the samples were placed in transport tanks, all dead organisms were removed, labeled to identify sample, their time and date of collection were recorded, and the organisms were preserved on ice for later examination. At approximately 1200 hours on the day following collection of the sample (about 21–27 h after collection), the remaining organisms were removed, their condition identified (living or dead), and the sample identification number and time and date of collection were recorded. All organisms collected in the sample were then identified, enumerated, and their total length (TL) was measured.

The effect of salt-box use was evaluated for all bycatch species and for Atlantic croaker alone. Three measurements were used for the evaluation: percent survival of bycatch organisms when separation of catch was completed (initial survival), percent survival of bycatch organisms at completion of sample observations (final survival), and mortality rate (M) of bycatch. Mortality rate was the absolute value of the slope of the regression of the natural log of living organisms +1 as a function of time. Time was measured from the time the catch was removed from the water to completion of sample observation. Because the three measurements had unequal variances, even when data were transformed, nonparametric procedures were used to compare the two separation methods and to examine relationships. Wilcoxon signed rank test was used to determine if initial survival, final survival, M, number of specimens collected, and TL of specimens differed significantly between the two separation methods. The Spearman correlation coefficient and the probability that the coefficient was significantly greater than 0 were used to examine relationships among all variables. For the correlation analysis, the use of a salt-box was coded as 1 and non-use as 0.

Stepwise multiple regression analysis was used as an exploratory procedure to examine the effect of 1) length of time trawl was fished; 2) catch separation time (total time bycatch was onboard the vessel); 3) bay temperature at the time of collection; 4) bay salinity at time of collection; and 5) effect of use or non-use of a salt-box on initial survival, final survival, and M of bycatch. For the regression analysis, coding of the use and non-use of a salt-box was the same as that for the correlation analysis. The significance level of the independent variable to enter and stay in the model was $P \leq 0.05$. Statistical analyses were performed by using the Statistical Analysis System (SAS Institute, 1990). All statistical tests were considered significant at the $P=0.05$ level.

Effect of salt-box use on bycatch survival: control experiments

Fourteen samples were collected from Matagorda Bay between 16 May and 25 October 1995, with a 6.1-m wide trawl constructed of 3.8-cm stretched nylon multi-filament mesh (Kana et al., 1993) equipped with a 6-mm stretch-mesh liner in the codend. The trawl was towed for 15 minutes. The catch was removed from the trawl and placed on a 0.67 × 1.0 m table. The catch was allowed to sit on the table for seven minutes to simulate the time the catch sits on deck while commercial shrimpers re-initiate trawling operations. The 7-min period was selected on the basis of a preliminary survey that we conducted of Texas bay shrimp fishing practices. The catch was then divided into approximately two equal portions: one half was placed in a salt-box and the bycatch was removed by skimming floating organisms from the surface; the remaining half of the catch remained on the table and shrimp were removed by hand, leaving the bycatch on the table. The salt-box was a 185-L fiberglass tank, half filled with bay water to which was added about 7.5 kg of food-grade salt. All bycatch removed from the salt-box was immediately placed in a separate 89-L transport tank filled with bay water and supplied with compressed oxygen. In the hand separation process, shrimp were removed and the bycatch remaining on the table was placed in a separate 89-L transport tank filled with bay water and supplied with compressed oxygen. Dead organisms were removed from samples immediately after they were placed in transport tanks. Samples were then transported to the PRB where they were treated in the same manner as previously described for samples collected from commercial shrimpers. The effect of the salt-box on the bycatch (all species), and on Atlantic croaker, spot, and sand seatrout (each species separately) was evaluated. Analyses were identical to those used to analyze samples collected from bay shrimpers.

Results

Bioassays

Probit analysis indicated LE_{50} for the five species ranged from a low of 17 min for red drum to 67 min for blue crab (Table 2). Southern flounder was the most tolerant of the fishes with an LE_{50} of 58 min. Comparison of log-likelihood ratio G values indicated that NOEE ranged eight min for Atlantic croaker and red drum to 64 min for blue crab. Southern flounder had the greatest NOEE (32 min) of the four fishes tested.

Effect of salt-box use on bycatch survival: samples from the bay shrimp industry

All commercial fishermen resumed fishing operations before beginning the catch separation procedure. Therefore, for descriptive purposes, catch separation time can be divided into two periods: 1) time the catch remains on deck or table while fishing is resumed and 2) time the catch is

Table 2

Results of high salinity bioassays and mean total length (TL) of experimental animals. LE_{50} = time required for 50% of experimental animals to die, NOEE = no-observed-effect from exposure time.

Species	n	LE_{50} (min)	NOEE (min)	Mean TL (mm)
Atlantic croaker	210	19	8	65 ±11.7
Red drum	105	17	8	28 ±2.7
Spotted seatrout	90	35	16	72 ±15.5
Southern flounder	105	58	32	131 ±23
Blue crab	105	67	64	45 ±4.8[1]

[1] Carapace width for blue crabs = TL.

Table 3

Mean (±SD) trawling time (h), catch separation time (min), bay salinity (‰), salinity in the salt-box (‰), and bay temperature (°C) for bycatch samples collected from the commercial fishery and separated with (n=15), or without (n=15), the aid of a salt-box and results of Wilcoxon signed rank tests comparing the means.

Variable	Salt-box	No salt-box	Z	P
Trawling time	1.5 ±0.62	1.2 ±0.79	0.634	0.526
Separation time	9.9 ±3.1	16.5 ±21.5	–0.605	0.550
Bay salinity	21.0 ±4.7	22.3 ±2.7	–1.045	0.296
Salt-box salinity	77.0 ±6.4			
Bay temperature	26.7 ±0.7	28.7 ±1.8	–3.887	<0.001

sorted. The mean time that catch remained on deck while fishing resumed was 11.7 min (±18.9 min) for fishermen not using a salt-box and 7.8 min (±4.2 min) for fishermen using a salt-box. Time the catch remained on the deck was statistically similar for the two methods (Z=0.6251, P=0.9502). The catch separation procedure with a salt-box began when fishermen used a large scoop to transfer the catch from the boat deck to the salt-box. Fishermen not using a salt-box spread the catch on the boat deck or a table and began removing shrimp. Once the catch separation procedure began, fishermen using a salt-box required 1.7 min (±1.4 min) to separate the catch, whereas fishermen not using a salt-box required 7.0 min (±12.0 min) to separate the catch; mean time required was statistically similar between the methods (Z=–1.44571, P=0.1483). Time required to separate the catch once the procedure began appeared to depend upon size of the catch, but we had only the captain's estimate of the size of the catch. Trawling time, catch separation time (total time bycatch was out of the water), and bay salinity at the time samples were collected did not differ significantly for samples collected by each method (Table 3). Mean bay temperature at the time of collection differed significantly between samples collected with the salt-boxes and those collected with no salt-box. Bay temperature averaged 28.7° (±1.8°)C when NSB samples were collected and 26.7° (±0.7°)C when SB samples were collected.

Thirty-three species (30 fishes and 3 invertebrates) were collected (Table 4). Atlantic croaker (n=536) was the most common organism and was present in 27 of the 30 samples. Other fishes frequently observed were spot (n=278), sand seatrout (n=90), Gulf menhaden (*Brevoortia patronus*, n=198), and bay anchovy (*Anchoa mitchilli*, n=45). The most frequently observed invertebrates were brown shrimp (*Penaeus aztecus*, n=46) and blue crab (n=34). Remaining species generally averaged <1 per sample. Some differences were observed in the frequency and length of some of the bycatch species that composed the NSB and SB samples. However, differences were generally small and were probably biologically insignificant. Atlantic croaker, bay anchovy, hardhead catfish (*Arius felis*), and brown shrimp were found in significantly greater numbers in SB samples than in NSB samples (Table 5). Bay whiff (*Citharichthys spilopterus*), bluntnose jack (*Hemicaranx amblyrhynchus*), Atlantic spadefish (*Chaetodipterus faber*), and inshore lizardfish (*Synodus foetens*) were found in significantly greater numbers in NSB samples than in SB samples, although they were rare in NSB samples. Mean number of specimens collected for all other species were similar in both sample types. Mean length of Atlantic croaker, Gulf menhaden, spot, sand seatrout, and pinfish (*Lagodon rhomboides*) were significantly greater in NSB samples (Table 5). Blue crabs collected in SB samples had a significantly greater mean carapace width than those collected in NSB samples. Mean lengths of all other species were similar in both sample types.

In general, all three measures of salt-box effect on bycatch were highly variable (Table 6). Mean (±SD) initial

Table 4

Species collected from bycatch of Texas bay shrimpers and in Texas Parks and Wildlife (TPW) samples. X indicates that the species was collected.

Common name	Species	Bay shrimpers	TPW
Vertebrates			
Atlantic bumper	*Chloroscombrus chrysurus*	X	
Atlantic croaker	*Micropogonias undulatus*	X	X
Atlantic cutlassfish	*Trichiurus lepturus*	X	X
Atlantic midshipman	*Porichthys plectrodon*	X	X
Atlantic spadefish	*Chaetodipterus faber*	X	X
Atlantic stingray	*Dasyatis sabina*	X	
Atlantic threadfih	*Polydactylus octonemus*		X
bay anchovy	*Anchoa mitchilli*	X	X
bay whiff	*Citharichthys spilopterus*	X	X
bighead searobin	*Prionotus tribulus*	X	X
blackcheek tonguefish	*Symphurus plagiusa*	X	X
bluefish	*Pomatomus saltatrix*	X	
bluntnose jack	*Hemicaranx amblyrhynchus*	X	X
gafftopsail catfish	*Bagre marinus*	X	X
gulf butterfish	*Peprilus burti*		X
gulf menhaden	*Brevoortia patronus*	X	X
hardhead catfish	*Arius felis*	X	X
harvestfish	*Perprilus alepidotus*	X	X
hogchoker	*Trinectes maculatus*	X	X
inshore lizardfish	*Synodus foetens*	X	X
least puffer	*Sphoeroides parvus*	X	X
lookdown	*Selene vomer*	X	
pinfish	*Lagodon rhomboides*	X	X
sand seatrout	*Cynoscion arenarius*	X	X
silver jenny	*Eucinostomus gula*		X
silver perch	*Bairdiella chrysoura*	X	X
skipjack herring	*Alosa chrysochloris*	X	
southern flounder	*Paralichthys lethostigma*	X	X
southern kingfish	*Menticirrhus americanus*	X	
spot	*Leiostomus xanthurus*	X	X
spotted seatrout	*Cynoscion nebulosus*	X	
star drum	*Stellifer lanceolatus*	X	
threadfin shad	*Dorosoma petenense*	X	
Invertebrates			
Atlantic brief squid	*Lolliguncula brevis*	X	X
blue crab	*Callinectes sapidus*	X	X
brown shrimp	*Penaeus aztecus*	X	X
white shrimp	*Penaeus setiferus*		X

survival rates were 76.1% (±21.7%) and 55.5% (±35.1%), respectively for SB and NSB samples. Final survival rates were 12.9% (±6.0%) for SB samples and 34.5% (±28.9%) for NSB samples. Means of initial and final survival were not significantly different. Average (±SD) M of organisms separated by use of a salt-box (0.08 [±0.03]) were significantly lower than M of bycatch separated without the aid of a salt-box (0.48 [±1.23]).

For individual species, comparison of the effect of use and non-use of a salt-box on M, and on initial and final survival variables could only be determined for Atlantic croaker. Other species were either found infrequently in individual samples, or in insufficient numbers within the sample type. Estimates of initial and final survival for Atlantic croaker were obtained from 19 samples (6 NSB samples and 13 SB samples). Estimates of M were obtained from 16 samples (4 NSB samples and 12 SB samples). Samples not used had too few Atlantic croaker (i.e. only one or two specimens in the sample or no deaths) to make estimates of percent survival or M. Means of the three estimates used to compare the two separation techniques were highly variable for Atlantic croaker (Table 6). Means

Table 5

Species which differed significantly in either mean numbers or total length (mm) from comparative samples collected from the commercial fishery and separated with or without the aid of a salt-box, and results of Wilcoxon signed rank tests used to compare means.

	Salt-box		No salt-box			
Species	Mean ±SD	*n*	Mean ±SD	*n*	*Z*	*P*
Number of specimens						
bay anchovy	2.7 ±4.9	15	0.3 ±0.6	15	3.159	0.002
Atlantic croaker	28.8 ±14.5	15	6.9 ±5.0	15	4.030	<0.001
hardhead catfish	2.5 ±3.3	15	0.7 ±1.4	15	2.150	0.032
bay whiff	0.1 ±0.3	15	1.3 ±1.4	15	–3.097	0.002
bluntnose jack	0	15	1.3 ±1.7	15	3.440	0.001
Atlantic spadefish	0	15	0.5 ±0.9	15	–2.366	0.018
inshore lizardfish	0	15	0.4 ±0.8	15	–2.073	0.038
brown shrimp	2.0 ±1.8	15	1.1 ±1.8	15	3.397	0.001
Total length						
Atlantic croaker	120 ±15	419	104 ±19	93	8.129	<0.001
gulf menhaden	110 ±47	120	119 ±22	57	3.460	<0.001
spot	98 ±23	157	109 ±20	60	3.910	<0.001
sand seatrout	103 ±30	45	129 ±25	44	4.830	<0.001
pinfish	75 ±13	9	89 ±20	82	–3.348	0.001
blue crab	82 ±23	60	66 ±28	16	–2.268	0.023

Table 6

Means of bycatch (all species) and Atlantic croaker initial percent survival, final percent survival, and mortality rate (*M*) and results of Wilcoxon signed rank tests comparing the means. All samples were collected from Texas bay shrimpers and separated with or without the aid of a salt-box.

	Salt-box		No salt-box			
Variable	Mean ±SD	*n*	Mean ±SD	*n*	*Z*	*P*
Bycatch						
initial survival	76.1 ±21.7	15	55.5 ±35.1	15	1.763	0.078
final survival	12.9 ±6.0	15	34.5 ±28.9	15	–1.389	0.093
M	0.08 ±0.03	15	0.48 ±1.23	15	2.605	0.009
Atlantic croaker						
initial survival	74.4 ±25.7	13	81.8 ±25.1	6	0.746	0.046
final survival	28.6 ±19.9	13	62.3 ±30.7	6	2.330	0.020
M	0.23 ±0.12	12	0.26 ±0.19	4	0.364	0.716

of *M* were similar between separation techniques. Means of initial (81.8% [±25.1%]) and final (62.3% [±30.7%]) survival of fish separated without a salt-box were significantly greater than initial (74% [±25.7%]) and final (28.6% [±19.9%]) survival of those separated with a salt-box.

By examining Spearman correlation coefficients that were significantly greater than 0, we found a relationship between the three measurements and factors other than use or non-use of a salt-box (Table 7). Initial and final survival estimates were negatively correlated to trawling time and catch separation time, whereas *M* was positively correlated to the two variables. Only estimates of *M* (r_s=0.488) were found to be related to the use or non-use of a salt-box. Final estimates of survival and *M* were also associated with some bycatch species. *M* was positively correlated to numbers of Atlantic croaker (r_s=0.526) and sand seatrout (r_s=0.458) in the samples, whereas final survival was inversely correlated to the two species (Atlantic croaker r_s=–0.391, sand seatrout r_s=-0.564). Other species to which *M* and final survival were associated occurred in fewer than half the samples or averaged <1 specimen per sample. Initial survival estimates were not related to

Table 7

Spearman correlation coefficients (r_s) and probability (P) that the coefficient values were greater than 0, of variables that demonstrated relationships with mortality rate (M), initial percent survival, and final percent survival for bycatch (all species) and Atlantic croaker. Samples were collected from the catch of Texas bay shrimpers and separated with or without a salt-box. A space indicates the probability that the variables r_s value was greater then 0 was >0.05.

	M		Initial survival		Final survival	
Variable	r_s	P	r_s	P	r_s	P
Bycatch						
separation time	0.638	<0.001	–0.586	0.001	–0.749	<0.001
trawling time	0.650	<0.001	–0.414	0.023	–0.665	<0.001
bay temperature	–0.459	0.011				
bay anchovy	0.477	0.008				
Atlantic croaker	0.526	0.003			–0.391	0.033
gulf menhaden	0.445	0.014			–0.461	0.010
sand seatrout	0.458	0.011			–0.564	0.001
pinfish	–0.434	0.017				
bighead searobin	–0.368	0.046				
gafftopsail catfish					–0.398	0.029
bay whiff					0.379	0.039
Atlantic cutlassfish					–0.373	0.042
salt-box	0.488	0.006				
Atlantic croaker						
separation time	0.637	0.008	0.459	0.016		
least puffer	0.994	<0.001				
hardhead catfish					–0.592	0.001
blue crab					–0.463	0.015
brown shrimp					–0.566	0.002
harvestfish			0.401	0.038	0.400	0.038
Atlantic brief squid	–0.833	0.039				
salt-box					–0.629	0.004

Table 8

Independent variables that entered stepwise multiple regression models describing bycatch (all species), initial percent survival, final percent survival, and mortality rate (M), and Atlantic croaker M resulting from Texas bay shrimpers separating them from shrimp with or without the aid of a salt-box. Included are y-intercepts±SE, coefficients±SE of the selected independent variables, partial r^2 of each variable in parenthesis, and model r^2. A space indicates the variable did not enter the model.

		Independent variable				
Dependent variable	y-intercept	Separation time	Trawl time	Bay temperature	Salt-box	Model R^2
Bycatch						
initial survival	217.4 ±65.3	–120.5 ±21.8 (0.52)				0.52
final survival	60.0 ±6.9		–20.2 ±4.1 (0.48)		–15.5 ±5.8 (0.11)	0.59
M	2.3 ±1.2	5.1 ±0.4 (0.79)	–0.3 ±0.1 (0.03)	–0.1 ±0.04 (0.03)		0.85
Atlantic croaker						
final survival	51.2 ±10.1	61.9 ±27.0 (0.11)			–31.22 ±10.0(0.38)	0.49

any bycatch species. The initial survival (r_s=0.459) and M (r_s=0.637) of Atlantic croaker were related to catch separation time. Atlantic croaker final survival (r_s=–0.629) was negatively correlated to use of a salt-box.

Stepwise multiple regression identified four variables, which explained most of the variation associated with the three measurements (Table 8). Catch separation time (total time that bycatch was on the vessel), trawling time, and

Table 9

Species collected from samples of bycatch separated with or without the aid of a salt-box in control experiments whose mean total length (mm) differed significantly and results of Wilcoxon signed rank tests used to compare the means.

	Salt-box		No salt-box			
Species	Mean total length ±SD	n	Mean total length ±SD	n	Z	P
Bay anchovy	50 ±11	191	53 ±13	219	–2.977	<0.001
Spot	94 ±18	138	89 ±24	194	2.818	0.005
Silver perch	72 ±37	4	111 ±22	18	–2.605	0.009
Atlantic brief squid	82 ±39	53	58 ±39	63	4.177	<0.001

bay temperature explained 85% of the variation associated with estimates of bycatch M. Catch separation time was the most important of the three, explaining 79% of the variation. Catch separation time explained 52% of the variation observed in initial survival and was the only variable to meet the $P \leq 0.05$ significance level required for entry into the model. Trawling time explained 48% of the variation observed in bycatch final survival and use or non-use of a salt-box explained the remaining 11%. For the dependent variable, Atlantic croaker final survival, the use or non-use of a salt-box explained 38% of the variation explained by the model and catch separation time explained an additional 11%. The five independent variables did not meet the $P \leq 0.05$ significance level required to enter or stay in stepwise regression models for Atlantic croaker initial survival and M.

Effect of salt-box use on bycatch survival: control experiments

Twenty-eight species were collected for the control experiments: 24 fishes and 4 invertebrates (Table 4). Bay anchovy (n=3794), Atlantic croaker (n=2048), spot (n=949), sand seatrout (n=291), and Gulf menhaden (n=148) were the most numerous organisms and were observed in more than half the samples collected. Remaining fishes averaged <1 individual per sample. Brown shrimp (n=494) and Atlantic brief squid (*Lolliguncula brevis*, n=133) were the most numerous invertebrates caught. By species, no significant differences were found between numbers of individuals in SB and NSB samples. Bay anchovy, spot, silver perch (*Bairdiella chrysoura*), and Atlantic brief squid collected in NSB samples were significantly larger than those collected in SB samples, but differences for the most part were small or the organism was present in small numbers (Table 9). Remaining species were similar in size in both NSB and SB samples. Catch separation time averaged 12 min (±6 min) for SB samples and 18 min (±18 min) for NSB samples and was significantly different (Z=2.05399, P=0.04). At the time samples were collected, bay temperature averaged 26.0° (±3.1°)C, bay salinity, 18,0‰ (±7.2‰), and salt-box salinity 66‰ (±8‰).

Survival rates and mortality rates were highly variable for bycatch separated by both methods. Mean initial survival rates were 48.4% (±39.7%) and 43.6% (±39.3%), respectively for SB and NSB samples. Final survival rates were 5.4% (±8.9%) for SB samples and 9.5% (±18.7%) for NSB samples. Mortality rates averaged 0.1 (±0.04) for SB samples and 0.1 (±0.05) NSB samples. All estimates of survival and mortality were statistically similar (Table 10). By species, estimates of initial survival, final survival, and M for Atlantic croaker, spot, and sand seatrout were also highly variable and were statistically similar between the two methods of bycatch separation.

Spearman correlation coefficients significantly greater than 0 demonstrated a relationship between bycatch (all species) catch separation time and the variables initial survival (r_s=0.445) and M(r_s=0.522). (Table 11). Initial survival was found to have an inverse relationship with temperature. All three measurements of the effect of salt-box use on bycatch were associated with the frequency of occurrence of some species in the bycatch. The variable M was positively correlated to Atlantic croaker (r_s=0.765), spot (r_s=0.681), and sand seatrout (r_s=0.792) numbers. Initial survival was positively correlated to Atlantic croaker (r_s=0.630), spot(r_s=0.530), and blue crab (r_s=0.494) numbers and was inversely related to the numbers of bay anchovy (r_s=–0.795) and Gulf menhaden (r_s=–0.466). Final survival was positively correlated to blue crab (r_s=0.631) numbers and inversely related to bay anchovy numbers (r_s=–0.655). Other species to which M, and initial and final survival were related occurred in fewer than half the samples and averaged <1 organism per sample. Atlantic croaker M was related to bay temperature (r_s=0.828), bay salinity (r_s=–0.513), and to the presence of Atlantic brief squid (r_s=–0.833). Neither Atlantic croaker initial nor final survival estimates were correlated to other variables. Spot and sand seatrout M, initial survival, and final survival were not correlated to any variable.

For all species, stepwise multiple regression identified catch separation time, bay salinity, and bay temperature at the time of sample collection as significant variables which explained some of the observed variation in M and final survival (Table 12). The salt-box variable did not enter any model. Catch separation time explained 27% of the variation that was accounted for by the model for the dependent variable M, and an additional 11% was explained by bay salinity at time of sample collection. Bay temperature and salinity at the time of collection explained 34% of variation observed in the final survival model. No variable met the $P \leq 0.05$ significance level for entry into regression models

Table 10

Mean (±SD) of initial percent survival, final percent survival, and mortality rate (*M*) and results of Wilcoxon signed rank tests used to compare the means of bycatch (all species), Atlantic croaker, spot, and sand seatrout from bycatch samples collected in control experiments and separated with or without the aid of a salt-box.

	Salt-box		No salt-box			
Variable	Mean ±SD	*n*	Mean ±SD	*n*	*Z*	*P*
Bycatch						
initial survival	48.4 ±39.7	14	43.6 ±39.3	14	–0.460	0.643
final survival	5.4 ±8.9	14	9.5 ±18.7	14	–0.322	0.748
M	0.10 ±0.04	14	0.10 ±0.05	14	–0.277	0.782
Atlantic croaker						
initial survival	41.1 ±38.2	8	56.0 ±40.1	8	0.790	0.430
final survival	18.3 ±24.1	8	16.8 ±34.1	8	–0.373	0.709
M	0.41 ±0.77	8	0.82 ±1.27	8	0.105	0.916
Spot						
initial survival	70.9±30.3	12	71.7 ±27.3	12	–0.206	0.837
final survival	24.9±34.8	12	21.5 ±36.4	12	–0.695	0.519
M	1.30±2.71	12	2.07 ±3.26	12	0.799	0.424
Sand seatrout						
initial survival	85.7 ±28.8	11	72.3 ±26.7	11	–1.242	0.214
final survival	29.2 ±38.0	11	14.1 ±20.0	11	–0.774	0.439
M	3.58 ±6.31	11	4.27 ±6.38	11	0.417	0.677

Table 11

Spearman correlation coefficients (r_s) and probability (*P*) that the coefficient values were greater than 0, of variables which demonstrated relationships with bycatch (all species) mortality rate (*M*), initial percent survival and final percent survival and Atlantic croaker *M*. Samples were collected in Texas Parks and Wildlife trawls and separated from shrimp with or without the aid of a salt-box during control experiments. A space indicates the probability that the variables r_s value was greater then 0 was >0.05.

	M		Initial survival		Final survival	
Variable	r_s	*P*	r_s	*P*	r_s	*P*
Bycatch						
separation time	0.522	0.004	0.445	0.018		
Atlantic croaker	0.765	<0.001	0.630	<0.001		
spot	0.681	<0.001	0.530	0.004		
sand seatrout	0.792	<0.001				
least puffer	0.692	0.039				
bay temperature			–0.4249	0.025		
salt-box salinity			–0.680	<0.001	–0.635	0.015
bay anchovy			–0.795	<0.001	–0.655	<0.001
gulf menhaden			–0.466	0.012		
bay whiff			0.444	0.018		
brown shrimp			0.433	0.021		
brief squid			–0.588	0.001		
blue crab			0.494	0.008	0.631	<0.001
silver perch			–0.386	0.042	–0.480	0.010
bluntnose jack			–0.568	0.002		
Atlantic croaker						
bay temperature	0.829	0.042				
bay salinity	–0.514	0.028				
Atlantic brief squid	–0.833	0.039				

Table 12

Independent variables which entered stepwise multiple regression models describing bycatch (all species), Atlantic croaker, spot, and sand seatrout short-term final percent survival, or mortality rate (M) (or both) resulting from their separation from shrimp with or without the aid of a salt-box during controlled experiments. Included are y-intercepts (±SE), coefficients (±SE) of the independent variables, partial R^2 of each variable in parenthesis, and model R^2. A space indicates the variable did not enter the model.

		Independent variable			
Dependent variable	y-intercept	Separation time	Bay salinity	Bay temperature	Model R^2
Bycatch					
final survival	216.59 ±58.069		−2.71 ±0.88 (0.13)	−4.68 ±2.08 (0.21)	0.34
M	0.12 ±0.03	0.09 ±0.03 (0.27)	−0.002 ±0.001 (0.11)		0.38
Atlantic croaker					
M	−5.36 ±203	2.25 ±0.45 (0.75)		0.21 ±0.08 (0.08)	0.83
Spot					
M	−1.44 ±1.41		0.18 ±0.07 (0.20)		0.20
Sand seatrout					
final survival	213.31 ±67.36			−7.08 (0.29)	0.29
M	−39.75 ±12.90			1.61 ±0.47 (0.37)	0.37

for the dependent variable initial survival. Time required to separate catch accounted for 75% of the variation observed in Atlantic croaker M, whereas bay temperature at time of sample collection accounted for the remaining 8% variation explained by the model. Bay salinity at time of sample collection explained 20% of the variation observed in M of spot. Bay temperature at time of sample collection explained 37% and 29%, respectively, of the dependent variables M and initial survival of sand seatrout. No variable met the $P \leq 0.05$ significance level for entry into models to explain initial survival of Atlantic croaker, spot, or sand seatrout, or final survival of Atlantic croaker or spot.

Discussion

Use of a salt-box to separate bycatch, as practiced by Texas shrimpers, had little or no effect on short-term survival of bycatch. Measurements of bycatch (all species) initial and final survival were not significantly different for the bycatch separation methods in samples collected from bay shrimpers and from TPW trawls. Significant differences between SB and NSB Atlantic croaker initial and final survival of samples collected from the commercial fishery were not corroborated by the results of the TPW control experiments. Exposure time to hypersaline conditions in a salt-box is short, generally less than two min, and the no-observed-effect exposure for selected species suggests that longer exposure (at least eight min for red drum and Atlantic croaker) would probably be required to significantly affect survival of most bycatch species. For these reasons, regulation of salt-box use as currently practiced by Texas bay shrimpers is unnecessary.

The use of a salt-box had little impact on bycatch M, and initial or final survival. Salt-box use was correlated to M, for samples collected from the fishery, but not to initial or final survival. Salt-box use was not correlated to any of the three variables for bycatch in the control experiments. Atlantic croaker final survival was negatively correlated to the presence of a saltbox in samples collected from the fishery but the correlation was not present in the control experiments. The salt-box variable entered the final survival regression models for combined catch and Atlantic croaker from the fishery but entered no other regression models.

Environmental factors at the time of sample collection played a role in determining survival of bycatch, although not as great as other variables associated with bycatch separation. Both bay temperature and salinity at the time of sample collection affected survival and M variables in the control experiments as demonstrated by the correlation and regression analyses. Bay temperature also explained a small portion of the variation (3%) associated with M in trials conducted on samples collected from the fishery. For individual species, bay temperature and salinity, either individually or combined, explained part of the variation associated with the regression models for M of Atlantic croaker, spot, and sand seatrout, and for final survival of sand seatrout. The effect of temperature on M in samples taken from the fishery and survival and M in control experiments suggest there may be a seasonal effect on survival of bycatch. Failure to observe a greater effect in trials with the fishery samples probably is due to the effect of other factors associated with the fishery, such as long trawl times and catch separation times that mask the effect of environmental factors.

Catch separation time was one of the most important variables associated with bycatch survival. It was correlated to M, initial survival, and final survival for samples collected from the fishery and to M and initial survival in the control experiment. The variable entered multiple re-

gression models for the variables M and initial survival of bycatch in experiments conducted with samples collected from the fishery. It also entered multiple regression models for the dependent variable M of bycatch and Atlantic croaker in the control experiments. The use of the salt-box significantly reduced catch separation time in control experiments, suggesting that the use of the salt-box could possibly reduce M by speeding catch separation time. The importance of reducing catch separation time has been corroborated by Ross and Hokenson (1997) who reported a positive correlation between mortality and on-deck sorting time for three species of bycatch (American plaice, *Hippoglossoides platessoides*; witch flounder, *Glyptocephalus cynoglossus*; and pollock, *Pollachius virens*) associated with the Gulf of Maine northern shrimp (*Pandalus borealis*) fishery.

Trawling time was also an important variable affecting M and survival. It was correlated to M, initial survival, and final survival of bycatch and entered the final survival regression model for bycatch from the fishery. This variable may affect survival in several ways. Longer trawl times in general result in larger catches. This would presumably increase catch separation time thus contributing to increased bycatch mortality. Long trawl times would also increase the time bycatch is in the net, thus increasing the chance of fatal injuries.

In the absence of a satisfactory method to reduce the catch of nontarget species, survival of bycatch could probably be improved by limiting the time a trawl is fished and by requiring the catch to be separated prior to resumption of fishing. These variables were the only ones found in our study that had significant impact on bycatch survival and that could easily be altered to improve survival. Enforcement of either of these proposals, however, would be difficult.

Use of percent survival estimates at the conclusion of the observation periods in our study to predict long-term survival of bycatch released into the bay is not recommended. Stress in fishes is characterized by hypersecretion of epinephrine, norepinephrine, and corticosteroids. These hormones induce secondary effects resulting in changes in metabolism, osmoregulation, and the immune systems (Mazeaud et al., 1977) that last for several days and can result in increased susceptibility to disease (Wedemeyer and McLeay, 1981). Furthermore, stress can lead to behavioral changes (Wedemeyer, 1974) that might make the fish more susceptible to predation. Specimens can be maintained under identical conditions (in the laboratory or field) to compare survival and mortality rates of individuals treated differently at capture. However, it would be speculative to project survival estimates of specimens maintained in any form of captivity to those released into the wild because it is impossible to account for predation and possibly disease.

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Trace metals in four structures of fish and their use for estimates of stock structure

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Abstract—Trace elements in calcified tissues have been suggested as one of the most powerful means for stock discrimination yet developed. The structure of choice for determining elemental composition or fingerprints is the otolith, although other structures also incorporate trace elements into their matrix. The aim of this study was to compare the elemental fingerprints of four structures (otoliths, scales, eye lenses, and spines) of a territorial reef fish to determine whether there were correlations between otoliths and each of the other structures. Elemental fingerprints of juvenile (<3 years of age) and adult fish (which may reach a maximum age of 37 years) were also compared for each structure to determine whether there may be differences between size classes of fish. All structures were analyzed by solution-based inductively coupled plasma-mass spectrometry (ICP-MS). Otoliths, scales, spines, and eye lenses differed in composition. Calcium dominated otoliths, scales, and spines but was not detected in eye lenses. Some elements, for example barium, showed significant correlations between the otolith data and that of scales and spines of both juvenile and adult fish. A multivariate test of matrix correspondence (Mantel's test) detected significant relationships between the otolith data and the data matrices for each of scales and spines of both juvenile and adult fish. Significant relationships between the otolith data and the eye lens data were detected only for juvenile fish. Significant differences were also found between juvenile and adult fish for all structures. The BIOENV multivariate analyses showed that the highest rank correlation was found between the otolith data and the scale or spine data for both juvenile and adult fish. These data suggest that the use of scales and spines may provide a nonlethal alternative to the use of otoliths for future stock discrimination studies.

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Studies of the population dynamics of marine fishes depend on the ability to distinguish separate "stocks" that may make up the total population of a single fish species. Although much of fisheries management assumes that a single population is being monitored, measures of growth, natural mortality, recruitment, and reproduction are often made for the total population because data on movement or stock separation or fidelity (or the combination of all three) are inadequate to allow separation of stocks. A variety of methods exist for identifying stocks (e.g. population parameters, capture-mark-recapture studies, physiological and behavioral characters, morphometric and meristic characters, calcareous characters, cytogenic characters, and biochemical characters; Ihssen et al., 1981), but few methods can provide a reliable measure of stock identity. Genetic techniques are frequently used but fail to differentiate stocks because a small amount of larval or adult mixing among populations makes differences undetectable (Hartl and Clark, 1989). Recently, many studies have focused on the use of "elemental fingerprints" (*sensu* Campana et al., 1994), or the elemental composition of the otoliths, as a measure of stock identity.

Initial microchemistry studies tended to focus on diadromous species and distinguished between freshwater and marine life history phases of fish (e.g. Bagenal et al., 1973; Belanger et al., 1987; Kalish, 1990; Coutant and Chen, 1993; Rieman et al., 1994). The electron microprobe was widely used for such studies but because of the "typical" concentrations of Ca, Na, Sr, K, S, and Cl found in otoliths, electron probes were effectively limited to detecting these six elements. Recently, there have been dramatic advances in analytical techniques and studies now indicate that other elements also exist in otoliths (e.g. Edmonds et al., 1989, 1991, 1992; Sie and Thresher, 1992), many of which are thought to be reflective of the environment (Fowler et al., 1995). Stock discrimination capabilities have improved with the sensitivity of recent instrumentation and the inductively coupled plasma-mass spectrometer (ICP-MS) has now become the instrument of choice for simultaneously quantifying the concentration of multiple elements and isotopes (Houk, 1986; Date, 1991).

ICP-MS has recently been used for assays of various fish tissues including otoliths (e.g. Edmonds et al., 1991; Campana and Gagne, 1995; Campana et al., 1994, 1995; Dove et al., 1996; Gillanders and Kingsford, 1996), scales (e.g. Coutant and Chen, 1993, Wells et al., 2000), eye lenses (e.g. Dove and Kingsford, 1998) and soft tissues (e.g. Beauchemin et al., 1988; Ishii et al., 1991; Hellou et al., 1992).

Sample-specific differences in elemental concentrations in tissues other than otoliths have also been reported (e.g. vertebrae—Mulligan et al., 1983; Behrens Yamada et al., 1987; scales—Bagenal et al., 1973; Lapi and Mulligan, 1981; eye lenses—Dove and Kingsford, 1998; soft tissue—Hellou et al., 1992). Such sample-specific differences in trace elements provide evidence for the nonmixing and segregation of populations. Otoliths are an ideal natural marker of elemental composition because they grow throughout the life of the fish and once the elements are de-

posited, they are unlikely to be resorbed or altered (Campana and Neilson, 1985). Recently, Dove and Kingsford (1998) found that eye lenses may be suitable for differentiating populations of fish because the eye lens has no efficient mechanism for removing ions from the tissue. Eye lenses also grow by the addition of protein-rich cells to their outer surface. Bone, scales, and other soft tissues (e.g. gills, liver, muscles) will reflect a variety of factors including composition during growth, but resorption and remineralization may occur in some species (Gauldie and Nelson, 1990). The temporal stability of the elemental concentrations in other tissues is therefore questionable (Campana and Gagne, 1995). In some species, however, the market value of the fish is likely to be reduced by removing otoliths and eye lenses; therefore, structures such as scales or spines may be easier to obtain. If scales and spines show similar elemental fingerprints (for fish caught at different locations) to otoliths and eye lenses, then their use in stock discrimination studies may be warranted. The use of scales and spines would also allow sampling without the need to kill the fish, with the result that broodstock could be kept alive and individuals from rare or endangered stocks could be sampled. With the exception of two studies (Dove and Kingsford, 1998; Wells et al., 2000), who compared elemental fingerprints of otoliths with eye lenses and scales, respectively, there have been no comparative studies of more than two structures.

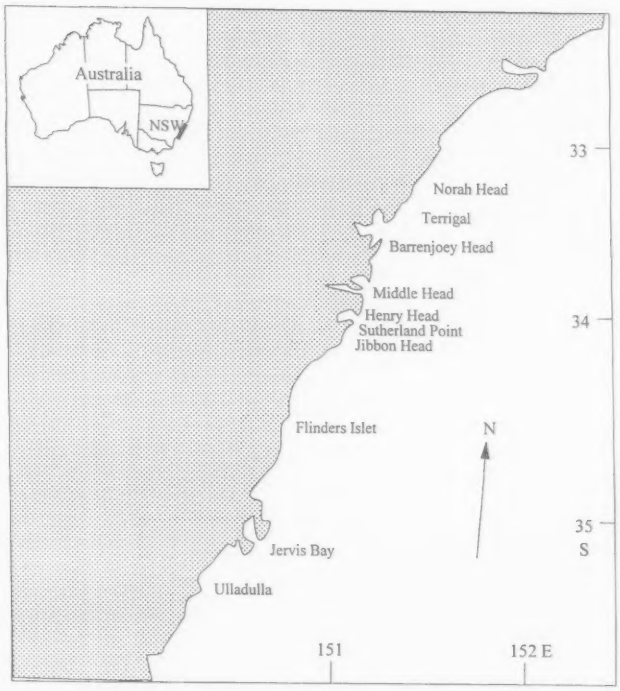

Figure 1

Map showing sampling locations along the coast of New South Wales, Australia.

The aim of my study was to compare the elemental fingerprints obtained from different structures (otoliths, scales, eye lenses, and dorsal spines) to determine if there were correlations among structures. Because otoliths are not subject to resorption (but see Mugiya and Uchimura, 1989), their use in studies of stock discrimination appears justified; therefore all comparisons were made between otoliths and the other three structures. These four structures chosen for the present study are also very different in terms of their composition. Otoliths are composed primarily of calcium carbonate, whereas scales and spines are composed primarily of calcium phosphate, and eye lenses, largely of water and structural proteins. There may, therefore, be differences in the affinities of ions for the different structures and this difference could have implications for the elemental chemistry of each structure.

The damselfish *Parma microlepis* is a territorial species, both juveniles (Moran and Sale, 1977) and adults (territory sizes 2–17 m^2, Tzioumis, 1995) remain attached to their natal sites. Therefore it is an excellent model species for this kind of research. Otoliths and eye lenses of *P. microlepis* have been shown to contain microconstituents that can be detected with ICP-MS, some elements of which may be site-specific (Dove et al., 1996; Dove and Kingsford, 1998). Juvenile fish are distinguished from adults on the basis of coloration and are generally less than 3 years of age (Tzioumis and Kingsford, 1999). By comparison adults reach a maximum age of 37 years, and fish larger than 120 mm SL may represent a wide range of age classes (Tzioumis and Kingsford, 1999). Adults will therefore have longer and potentially more variable age-integrated elemental fingerprints than those of juvenile fish.

Materials and methods

Parma microlepis was collected from each of ten locations along the coast of New South Wales, Australia (Fig. 1). Fish were not collected from multiple sites within each location because small-scale differences in trace elements of otoliths and eye lenses (sites separated by 50 to 200 m) were not found in a previous study (Dove and Kingsford, 1998). All fish were collected by divers using SCUBA and a hand-spear between January and April 1998. Between five and seven replicate adult and juvenile fish were collected from each location. Juveniles and adults were distinguished from each other on the basis of differences in patterns of coloration.

Fish were stored on ice during transportation to the laboratory. In the laboratory, the standard length of each fish was measured (Table 1). Otoliths, eye lenses, and approximately five non-regenerated scales were removed from each fish and rinsed in MilliQ water. Otoliths and scales were stored dry at room temperature, and eye lenses were stored at –70°C in Eppendorf microcentrifuge tubes in preparation for ion analysis. Dorsal spines were removed and frozen (–4°C) prior to removal of surrounding flesh. After flesh was removed, spines were also stored dry at room temperature.

Table 1

Summary of collections of *Parma microlepis*. Sample locations are shown in Figure 1. Sample sizes were n=5 for juveniles and adults at all locations, except for adult fish at Henry Head where n=7. Lengths refer to standard length in mm.

Sample no.	Location	Date of collection	Juvenile mean length (range)	Adult mean length (range)
1	Norah Head	Mar 1998	72.0 (56–89)	125.6 (117–133)
2	Terrigal	Mar 1998	83.0 (61–95)	122.6 (105–132)
3	Barrenjoey Head	Feb 1998	64.0 (49–89)	118.8 (106–129)
4	Middle Head	Feb 1998	88.6 (83–94)	125.6 (115–136)
5	Henry Head	Jan 1998	83.6 (71–94)	122.6 (114–136)
6	Sutherland Point	Jan 1998	85.4 (76–93)	118.6 (107–125)
7	Jibbon Head	Mar 1998	83.2 (65–95)	126.4 (114–136)
8	Flinders Islet	Mar 1998	76.0 (73–80)	110.4 (105–124)
9	Jervis Bay	Apr 1998	81.2 (75–95)	117.8 (112–125)
10	Ulladulla	Apr 1998	80.8 (76–90)	111.2 (102–120)

All samples and standards were prepared for ion analysis in a laminar flow cabinet. Otoliths and eye lenses, and scales and spines were individually weighed on an analytical balance (to 0.0001 g) and a microbalance (to 0.001 mg), respectively. All samples were rinsed in 1% nitric acid for 15 s prior to dissolution. Structures were digested in nitric acid (Aristar) either overnight (otoliths and scales) or for 36 h (eye lenses and spines). For eye lenses, sulphuric acid (Aristar) was added following dissolution and each sample was heated to ~90°C for 1 hour. For all structures varying amounts of MilliQ water were then added followed by additional dilution with 1% nitric acid where necessary. This was necessary so that concentrations of the sample solutions were standardized before analysis with ICP-MS.

Samples were analyzed by solution-based ICP-MS (Perkin Elmer SCIEX ELAN 5000). Initially, the four structures of fish collected from one location (Henry Head, Fig. 1) were analyzed in "totalquant II" mode (n=7 fish). This procedure provided a rapid-survey-analysis to semiquantitatively determine the elements present (and their range) within each of the four structures. The instrument was calibrated at six points spanning the mass range from 9 to 209. Accuracy and drift of the machine were determined from spiked samples.

Samples were then analyzed in "quantitative analysis" mode after the instrument was calibrated for either external standardization or addition calibration, depending on the structure and expected concentration range of each element. Measurement parameters for the ICP-MS were as detailed in Gillanders and Kingsford (1996). Seven blank solutions were run at the beginning of each session to determine detection limits, which were calculated from the concentration of analyte, yielding a signal equivalent to three times the standard deviation of the blank signal. Blank solutions were 1% HNO_3 for otoliths, scales, and spines, and 2% acid (1%HNO_3 and 1%H_2SO_4) for eye lenses. Standards were run through the machine at the beginning of each session. Spiked samples were run every ten samples to measure sensitivity changes to the machine through use and for recovery verification.

All samples were initially analyzed by using addition calibration mode. The data were then reprocessed in external calibration mode to obtain concentrations of microelements. Each sample took around 3 to 4 min to analyze, including a delay before starting to read a sample (70 s) and the rinse (1% HNO_3, 0.1% Triton X) between samples of 60 s. Samples from each structure (otoliths, scales, spines, and eye lenses) and size class (juveniles and adults) were analyzed together in blocks, but all samples within each block were randomized to ensure that resulting patterns did not reflect variation in the performance of the instrument.

Univariate and multivariate techniques were used to test hypotheses concerning individual elements and multielement fingerprints of *Parma microlepis*. Pearson's correlations were used to determine the relation between otoliths and each of the other structures for individual elements (Mn, Sr, Ba, and Pb). Separate analyses were performed for each size class and structure.

The match between the otolith microchemistry data and the microchemistry data for each of the other three structures was examined by using two types of nonparametric multivariate analysis. For both types of analysis, the data were standardized by subtracting the mean and by dividing by the standard deviation of the variable, so that each element would have equal weight. A matrix of Euclidean distances among all pairs of samples was then calculated. Mantel's test (from the R package) and the BIOENV procedure (included in the PRIMER computer program; copyright M.R. Carr and K.R. Clarke, Marine Biological Laboratory, Plymouth, UK) were used to determine the degree of correlation between pairs of distance matrices (otoliths and each of the other three structures).

Mantel's test describes the relation between two distance matrices with Pearson's correlation coefficient, r (Mantel, 1967; Legendre and Fortin, 1989). A test of significance for r is obtained by random permutations of the replicate sample units for one of the matrices. A total of

999 permutations were performed for each test and the probability calculated as the number of values equal to, or larger than, the observed value of r divided by the total number of permutations.

If significant relationships were found with Mantel's test, the BIOENV procedure was then used to determine which elements were likely to be important in describing the correlation between the distance matrices (Clarke and Ainsworth, 1993; Clarke and Warwick, 1994). BIOENV calculates rank correlations between a dissimilarity matrix derived from the otolith microchemistry data and matrices derived from various subsets of the elements for either the scale, spine, or eye lens data matrices, thereby defining suites of elements that "best explain" the otolith microchemistry data (Clarke and Ainsworth, 1993). The harmonic or weighted Spearman correlation ρ_w was used.

Results

Otoliths, scales, spines, and eye lenses of *Parma microlepis* differed in composition, both in terms of the actual elements present and the concentration of individual elements. Otoliths, scales, and spines were dominated by calcium, whereas no calcium was detected in eye lenses. The microelement Sr was present in otoliths, scales, and spines at concentrations of 100's to 1000's µg/g of structure, whereas it occurred at low concentrations in eye lenses. Other elements (e.g. Mn, Ba, Pb) were typically found in low-to-trace levels in all structures. Mercury was found in detectable concentrations only in eye lenses.

Univariate analyses

Differences in the concentration of manganese among structures varied over several orders of magnitude (range: 0.05 for eye lenses to 100 µg Mn/g structure for spines). Concentrations of Mn in otoliths showed significant correlations with concentrations of Mn in both eye lenses and scales of juvenile fish, but only with scales from adult fish (Fig. 2, Table 2). For juveniles, fish from one site may have influenced the correlation analyses; therefore fish from this site were removed and the correlations recalculated. This adjustment resulted in a significant correlation between Mn in otoliths and Mn in scales, as found previously. The relation between Mn in otoliths and Mn in spines was also significant when fish from this one site were removed (r=0.302, P<0.05). However, there was no longer a significant correlation for eye lenses: fish from one site may therefore have influenced the correlation result for this structure.

Concentrations of Sr in otoliths showed significant correlations with concentrations of Sr in all the other structures for juvenile fish; however the relation was only positive for otoliths and spines (Fig. 3, Table 2). For scales and spines, fish from one site did not unduly influence correlations; when these fish were removed from analyses, correlations were still significant. However, as found for Mn, the correlation between Sr in otoliths and Sr in eye lenses was no longer significant when fish from this site were removed. The only significant relationship for Sr in adult fish was found between otoliths and spines (Fig. 3, Table 2).

Table 2

Correlations between concentration of elements in otoliths and concentration of elements in eye lenses, scales, and spines of juvenile and adult fish collected from ten locations along the coast of New South Wales. Shown are Pearson's correlation coefficients. Significance levels are indicated by * (P<0.05) and ** (P<0.01), df=48. No corrections were made for experiment-wise error rate; therefore 1 in 20 tests would be expected to be significant by chance alone.

	Otolith			
	Mn	Sr	Ba	Pb
Juveniles				
Eye lenses	0.3410*	–0.3787**	0.1260	
Scales	0.4018**	–0.6237**	0.8810**	
Spines	0.2466	0.6586**	0.9001**	
Adults				
Eye lenses	0.0429	0.2353	0.0239	–0.2564
Scales	0.4717**	–0.0051	0.8839**	–0.1487
Spines	0.2696	0.8446**	0.9121**	–0.0732

Significant positive relationships were found between the amount of Ba in otoliths and the amount of Ba in scales and spines of both juvenile and adult fish (Fig. 4, Table 2). Lead was usually at or below detection limits of the instrument for juvenile fish and therefore correlations were not made. Correlations between the amount of Pb in otoliths of adult fish and the other structures showed no significant relationship (Fig. 5, Table 2).

Multivariate analyses

Mantel's test detected a significant relationship between the Euclidean distances among replicates based on the otolith data and the distances based on data from eye lenses, scales, or spines for juvenile fish (Table 3). For adult fish, however, a significant relation was detected between the otolith data and the scale and spine data (Table 3); thus there may be either changes in assimilation of elements with age or resorption and remineralization may occur in some structures, for example in eye lenses.

The BIOENV analyses showed that the highest rank correlation was found between the otolith data and the scale data for juvenile fish and involved Sr and Ba (Table 3). For adult fish the highest correlation was between the otolith data and the spine data and involved three elements (Mn, Sr, and Ba; Table 3).

Comparisons of juvenile and adult fish for each structure showed no significant relationships (Table 3). The Mantel test, however, was marginally nonsignificant for otoliths (Mantel r statistic 0.2000, P=0.060).

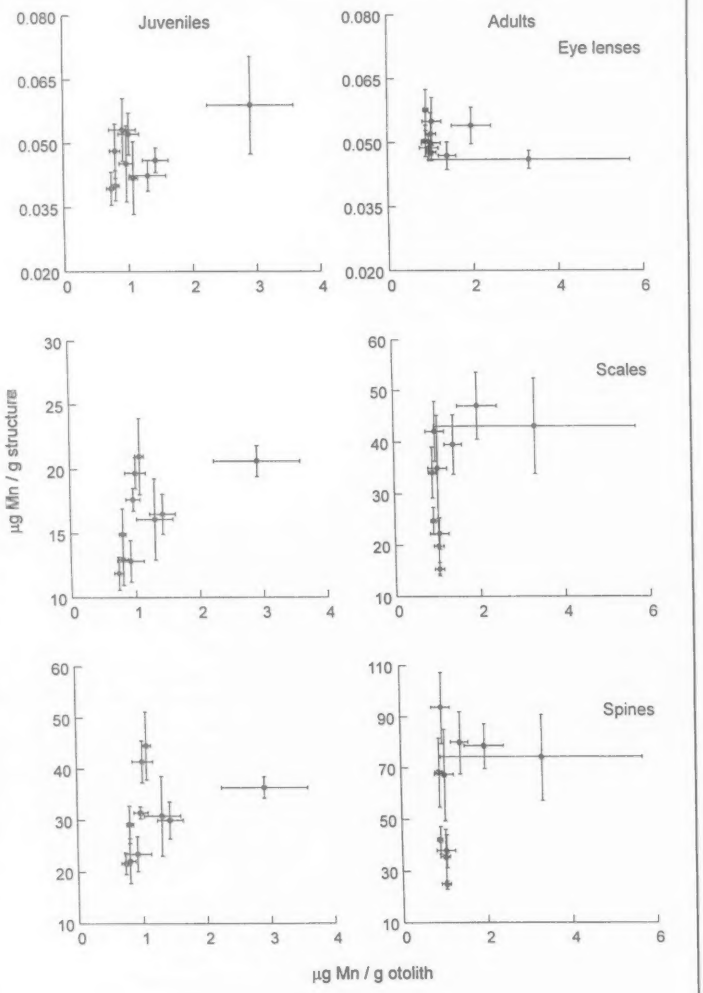

Figure 2

Relation between concentration of manganese in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

Discussion

Otolith elemental fingerprints are reported to be among the most important tools for stock discrimination for some species of fish (Campana et al., 1995). In the current study, adult fish showed a significant relationship between the otolith elemental fingerprints and the scale and spine elemental fingerprints. In contrast, juvenile fish showed significant relationships between comparisons of otoliths and each of scales, spines, and eye lenses. Relationships among some structures were also seen for some individual elements. There have been two other studies involving comparisons between elemental fingerprints of otoliths and other structures (Dove and Kingsford, 1998; Wells et al., 2000). Wells et al. (2000) compared trace elements in otoliths and scales of juvenile weakfish by using correlation analyses on individual elements. They found similar results to those in the current study, namely a significant correlation between Mn, Sr, or Ba in otoliths and that in scales. Dove and Kingsford (1998) compared elemental fingerprints of otoliths and eye lenses. In their study differences between locations were compared by using analysis of similarity (ANOSIM) permutation tests. Where comparisons between locations gave the same response

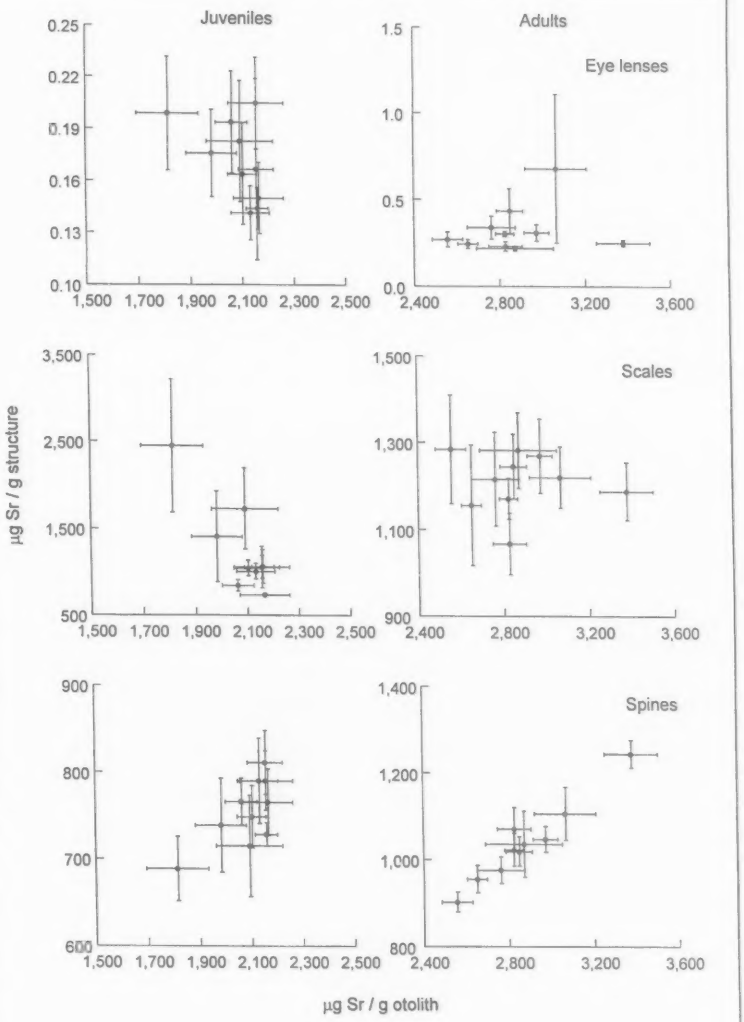

Figure 3

Relation between concentration of strontium in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

for both otoliths and eye lenses (either both significant or both nonsignificant), then the two structures were viewed as being similar. With this approach, approximately 73% (11/15) of pair-wise comparisons showed agreement (Dove and Kingsford, 1998). Their approach is, however, indirect and does not show evidence of correlation between data matrices for otoliths and those for eye lenses. I am aware of no other studies that have compared multiple structures for fish obtained from a number of locations.

Otoliths have been considered the structure of choice for determining elemental fingerprints and for relating these to movements or stock structure of fish because otoliths grow throughout the life of the fish and the material of the annual growth increment, once deposited, is unlikely to be resorbed or altered (Campana and Neilson, 1985). Otoliths therefore have the potential to record endogenous and exogenous factors permanently within their calcium-protein matrix. Other structures may also grow throughout the life of the fish (e.g. eye lenses—Dove, 1997; scales—Mitani, 1955; spines—Hill et al., 1989). Although other structures have been used for chemical analyses and for aging fish, some structures are susceptible to resorption and remin-

Table 3

Summary of results from Mantel's test and the BIOENV procedure where otolith microchemistry data were compared with the microchemistry data obtained from eye lenses, scales, and spines. Comparisons are also made between juvenile and adult fish for each structure. The BIOENV procedure (see general text) was done only if significant correlations were found with Mantel's test. Only the combination of elements that contributed to the maximum ρ overall, as measured by the weighted Spearman correlation, are shown for each structure comparison. The significance of the Mantel test statistic is shown by * ($P<0.05$) or ** ($P<0.01$).

Comparison	Mantel r statistic	BIOENV-maximum ρ	Combination of elements contributing to maximum ρ
Juveniles			
Otoliths and eye lenses	0.3402*	0.127	Mn, Ba, Hg
Otoliths and scales	0.5757**	0.587	Sr, Ba
Otoliths and spines	0.4097**	0.403	Sr, Ba
Adults			
Otoliths and eye lenses	–0.0646		
Otoliths and scales	0.3680**	0.454	Mn, Ba
Otoliths and spines	0.4440**	0.512	Mn, Sr, Ba
Adults and juveniles			
Otoliths	0.2000		
Eye lenses	–0.1357		
Scales	–0.0136		
Spines	0.0676		

eralisation (Simkiss, 1974). Despite the possibility of resorption of some elements, otoliths and scales, and otoliths and spines showed a significant correspondence between data matrices for both juvenile and adult fish, which may indicate that scales and spines provide estimates of stock structure that are similar to those obtained from otoliths.

Differences in elemental fingerprints among structures may be due to different routes of ion uptake and differential abilities of each structure to incorporate elements into the organic and inorganic matrix. Calcium and strontium in otoliths are primarily taken up by the gills (Simkiss, 1974), but the route of uptake of trace elements has not been identified (Campana and Gagne, 1995). There may also be some Sr uptake in otoliths through the diet because fish that were fed a Sr-enriched diet showed a detectable increase in Sr/Ca ratios (Gallahar and Kingsford, 1996). Other structures, such as scales and bone, may incorporate ions by diffusion across the gills and through the skin or by ingestion of food and water (Simkiss, 1974). Whether some structures show differential abilities to incorporate ions through different methods of absorption and whether ions are resorbed from different structures in equal proportions is not known.

Otoliths and eye lenses showed the greatest differences in elemental fingerprints between structures. Otoliths are predominantly $CaCO_3$ (Ca constitutes from 30% to 39% of otoliths, Thresher et al., 1994; Dove et al., 1996), although small amounts of protein also occur (Degens et al., 1969) and therefore otoliths are likely to incorporate ions that are able to substitute for Ca in the $CaCO_3$ matrix or bind to proteins in the organic matrix (Gunn et al., 1992; Sie and Thresher, 1992). The spaces between these matrices may also trap ions. In contrast, eye lenses are composed largely of water and structural protein, the latter of which may be soluble or insoluble crystallin (Nicol, 1989). The proteins of eye lenses are rich in sulfhydryl groups that may covalently bind with metals and the proteins also have specific sites for binding with cations (Sharma et al., 1989). Scales and bone are more similar to otoliths in that the dominant ion is Ca (e.g. in bone Ca may constitutes from 24% to 37%; Hamada et al., 1995), but they vary considerably in that otoliths are primarily carbonate structures, whereas scales and bone are primarily phosphate (or hydroxyapatite) structures. Some differences in elemental composition of otoliths and scales or spines may therefore be expected. Because otoliths, scales, and bone are composed predominantly of a mineral, rather than an organic matrix, it is not surprising that these structures were similar in their elemental fingerprints and that eye lenses presented a different fingerprint.

Differences in elemental fingerprints between juvenile and adult fish were also found for all structures. Eye lenses are thought to have no efficient mechanism for removing ions, but there may be changes in structural proteins with age that possibly alter affinities of proteins for specific ions (Dove, 1997). Such ontogenetic effects may result in different elemental fingerprints between juvenile and adult fish for eye lenses. Both scales and spines show some evidence of resorption or remineralization over time in some species. For example, early growth increments in spines of several species are known to be destroyed as the core-matrix expands (Hill et al., 1989; Gillanders et al., 1997) and there is evidence for resorption in scales of fish living in a stressed environment (Sauer and Watabe, 1989).

If an alternative structure to otoliths is required for stock identification, as it may be for broodstock, and for rare or endangered species, or if removing otoliths decreases

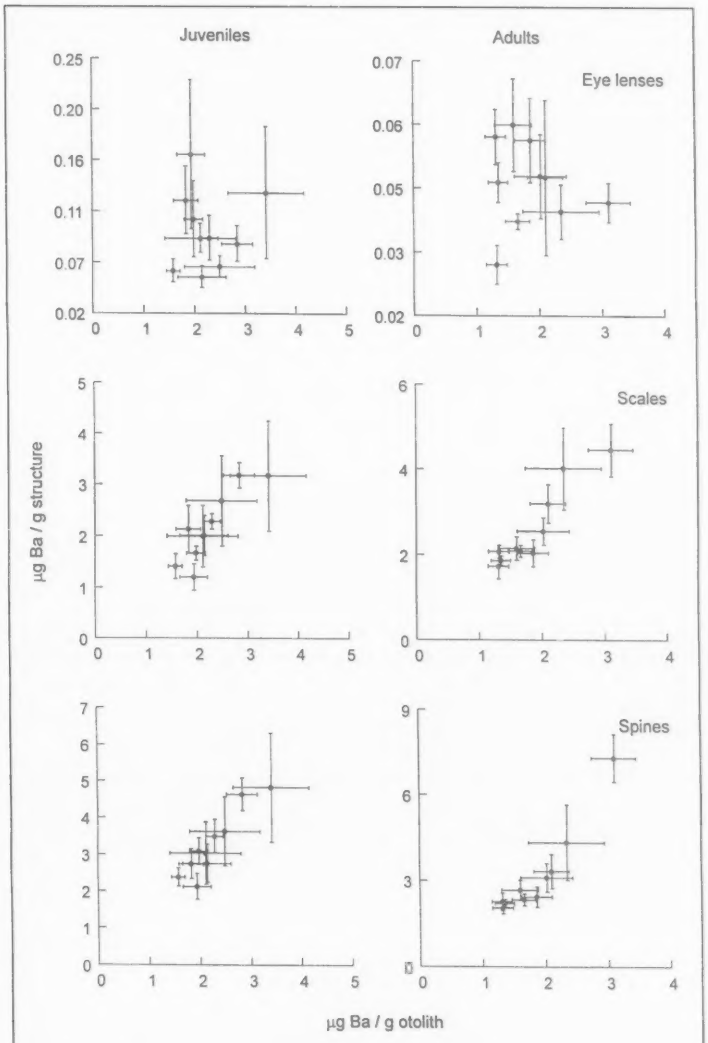

Figure 4

Relation between concentration of barium in otoliths (mean ±SE) and in eye lenses, scales, and spines of juvenile and adult fish (mean ±SE) collected from ten locations along the coast of New South Wales.

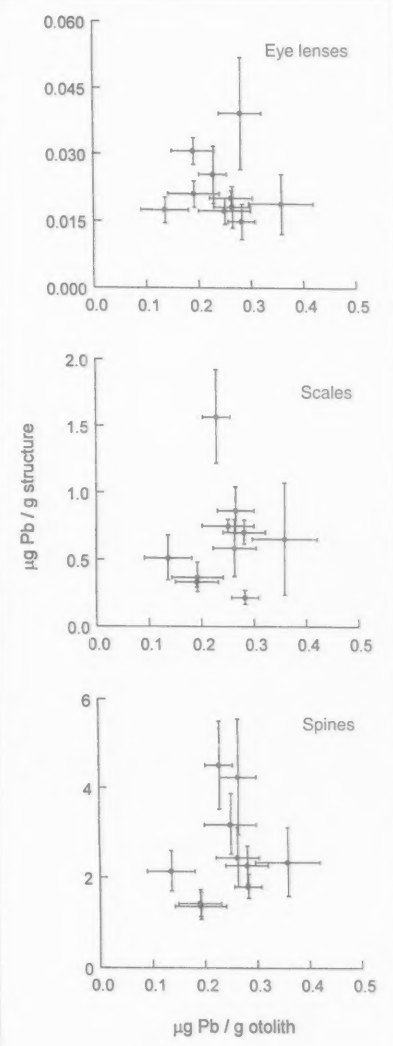

Figure 5

Relationship between concentration of lead in otoliths (mean ±SE) and in eye lenses, scales, and spines of adult fish (mean ±SE) collected from ten locations along the coast of New South Wales. Juveniles are not shown because concentrations of lead were below detection limits.

the market value of the fish, then it is recommended that either scales or spines are the best alternative structure because these were the structures that were significantly correlated with the otolith elemental data for both juvenile and adult fish. However, before using either of these structures, a number of fish (e.g. 30–50) should be collected from three to four locations and the alternative structure, as well as otoliths, should be analyzed. Classification models should then be developed by using data from each structure and the error rates should be compared. This was beyond the scope of the present study because sample sizes from each location were relatively small.

Scales and spines may offer certain advantages over otoliths because they provide a nonlethal alternative. In addition, they can be collected relatively quickly and easily. Scales may also require less preparation for analyses than either spines or otoliths. Thus, scales and spines may provide an alternative structure for determining stock identity.

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Estimating the number of fish in Atlantic bluefin tuna (*Thunnus thynnus thynnus*) schools using models derived from captive school observations

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Abstract—Aerial photographic assessment is a promising technique that could be structured to yield a fishery-independent index of abundance for Atlantic bluefin tuna, *Thunnus thynnus thynnus* (ABT). The accuracy of this approach may be increased by incorporating the relationship between the surface characteristics of a school and the total number of individuals. Our objective was to develop models to facilitate the estimation of number of fish in ABT schools from aerial photographs.

Video cameras were used to observe 74 incidences of schooling for 50 captive ABT approximately one meter in length. Relationships between the surface characteristics of ABT schools and the number of fish in the school were explored by using least-squares regression. The schools ranged in number from 2 to 45 individuals. A weighted regression model incorporating the number of fish in the school at the surface as the independent variable and the number of fish in the remaining portion of the school yielded an r^2 of 0.74. A second weighted multiple-regression model incorporating the number of fish in the school at the surface and in the second depth interval (0–25% school depth below surface layer) of the school as independent variables, and the number of fish in the remaining portion of the school as the dependent variable, with 1/variance as the weight, achieved an r^2 of 0.70. A third model using the length and width of the surface layer of the school as the independent variables and the number of fish in the school as the dependent variable had an r^2 of 0.86. One data point from a wild school is currently available to verify model predictions. This school of 125 individuals is well outside the range of school sizes used to construct the model (2–45 individuals), yet differs from model predictions by only 7%.

We believe that these models have the potential to improve an abundance index based on aerial photographs by estimating the number of individuals in wild ABT schools from surface characteristics observed in aerial photographs.

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Fish. Bull. 99:420–431 (2001).

The bluefin tuna (*Thunnus thynnus*) is distributed worldwide in temperate and subtropical seas. It has a limited distribution in the southern hemisphere. Endothermy by means of vascular heat exchangers allows bluefin tuna to inhabit a wide thermal niche and therefore wide geographic and depth ranges (Carey and Teal, 1969; Carey and Lawson, 1973). In the western Atlantic Ocean, the Atlantic bluefin tuna (ABT), *Thunnus thynnus thynnus*, is distributed from Labrador to Brazil, including the Gulf of Mexico and Caribbean Sea. Adult ABT occur throughout the entire range, but smaller bluefin tuna (less than 45 kg) are not observed frequently above the latitude of Cape Cod, Massachusetts. The Atlantic bluefin tuna is epipelagic and usually oceanic but appears near the coast seasonally (Squire, 1962; Collette and Nauen, 1983) to feed on concentrated assemblages of prey. Adult ABT may attain a length of four meters and a body mass of 680 kg. Large medium (178–195 cm FL) and giant (>195 cm FL) bluefin tuna are targeted by commercial purse-seine, long line, and hook-and-line fisheries (Mather, 1974; Figley, 1984). A recreational hook-and-line fishery (Mather, 1974; Figley, 1984) targets all sizes of bluefin tuna as they appear along the east coast of the United States and Canada from June to October (Mather, 1962).

The combination of changes in the spatial distribution over time and associated uncertainty regarding the independence of eastern and western Atlantic stocks makes the estimation of ABT stock size particularly problematic. The stock assessment for this species has been based upon landings data and abundance indices (Scott et al., 1993). Use of ABT landings data to generate an abundance index may lead to bias due to variability in effort, improvements in fishing technology (Lo et al., 1992), and variability in annual geographic distribution linked to prey distribution.[1] These characteristics of the northwest Atlantic bluefin tuna fishery, in conjunction with population-level behavioral characteristics observed for similar tuna species, suggest that the use of catch-per-unit-of-effort (CPUE) data to evaluate tuna population trends could lead to inaccurate estimates (Clark and Mangel, 1979). The accuracy of CPUE-based assessments in estimating the abundance of bluefin tuna in the northwest Atlantic remains controversial (Clay, 1991; Suzuki and Ishizuka, 1991; Safina, 1993). An extensive discussion of the issues involved in Atlantic bluefin tuna assessment can be found in the National Research Council report by Magnuson et al. (1994).

Recent investigations have focused on the feasibility of using aerial photographic assessment of large medium and giant bluefin tuna in New England waters and in the Straits of Florida (Lutcavage and Kraus, 1995; Lutcavage et al., 1997) as an alternative fishery-independent method of obtaining indices of abundance. The aerial survey

[1] Chase, B. C. 1995. Preliminary report of the Massachusetts bluefin tuna investigation: the diet of bluefin (*Thunnus thynnus*) off the coast of Massachusetts. Massachusetts Division of Marine Fisheries. Salem, MA 01947, 39 p.

method has been used to determine the relative abundance for other pelagic fisheries worldwide, including *Engraulis mordax* (Lo et al., 1992), *Engraulis mordax*, *Sarda chiliensis*, *Trachurus symmetricus*, etc. (Squire, 1972), *Trachurus declivis*, *Katsuwonus pelamis*, *Arripis trutta*, *Thunnus maccoyii* (Williams, 1981), *Mugil* spp. (Scott et al., 1989), and Squire (1993) has reported aerial survey data for *Thunnus thynnus orientalis* and other species. Abundance estimates derived from an aerial assessment are based on biomass or number of individuals per unit of area.

Lutcavage and Kraus (1995) concluded that the aerial method could provide area-specific minimum abundance and distribution data for large medium and giant Atlantic bluefin tuna under good viewing conditions. However, many difficulties associated with aerial photographic assessment of ABT remain to be resolved. Sea state, lighting conditions, and turbidity all play an important role in the ability to detect and produce useful photographs of schools (Lutcavage and Kraus, 1995). Rough seas, sun glare, and high turbidity may all result in reduced detection of schools, limiting the days on which this type of survey method is effective. Visual counts of individuals at the surface derived from aerial photographs are difficult to interpret without a verification count and information on behavioral factors such as surfacing frequency (Lo et al., 1992) and the proportion of the school visible at the surface (Lutcavage and Kraus, 1995). In addition, variability in population movements and distribution could lead to an inaccurate abundance estimate if an intensive, spatially expansive sampling scheme is not employed.

We propose a technique to address the problem of estimating the number of fish in a school (NFS) from the surface characteristics of a school. If the relationship between the surface structure or the surface number of fish and number fish in total school was known, school surface counts from aerial photographs or visual observations could be adjusted to include an estimate of total NFS, facilitating an improvement of area-specific minimum abundance estimates based on visual or photographic data sources.

Atlantic bluefin tuna are believed to exhibit the most rigidly defined spatial structure of schooling fishes (Partridge et al., 1983). Distinct two- and three-dimensional school structures have been described by previous authors (Partridge et al., 1983; Lutcavage and Kraus, 1995). Parabolas and echelons are the shapes of commonly observed surface-oriented two-dimensional schools, whereas the densely packed dome is the shape of a frequently observed three-dimensional school configuration (see Partridge et al., 1983 and Lutcavage and Kraus, 1995 for illustrations). The number of fish observed in two-dimensional surface schools is generally less than 15, whereas three-dimensional schools such as those forming densely packed domes usually have greater than 15 individuals (Partridge et al., 1983). Although the three-dimensional component of bluefin tuna school structure has been observed (Lutcavage and Kraus, 1995), quantitative description and analysis is lacking and little is known of the relationship between the two-dimensional surface structure and three-dimensional structure (e.g. total count, biomass) of schools (Partridge et al., 1983; Lutcavage and Kraus, 1995). In addition, the behavioral and environmental factors that may influence tuna school structure and dynamics remain poorly described (Mather, 1962; Clark and Mangel, 1979; Partridge et al., 1983).

Our study presents a functional relationship between the surface characteristics of and the total number of individuals in ABT schools. We analyzed video-taped footage of 74 incidences of schooling in a group of captive ABT to quantify the relationship between the number of fish visible at the surface and the total number of individuals in the school (NFS), the relationships between school dimensions (e.g. length, width) and NFS, and to explore the effect of environmental conditions within the net-pen enclosure on school size and dimensions. We also analyzed the vertical distribution of individuals within schools across school size, and propose a mechanistic explanation for the limited size of the two-dimensional schools observed by Partridge et al. (1983) and Lutcavage and Kraus (1995). We then apply the predictions from one of the resultant models to the single open-ocean school size estimate available.

Methods

Field methods

We employed a 30.5-m diameter, 15.3-m deep, cylindrical floating net-pen enclosure (Fig. 1) to hold the tuna used in our study. This enclosure is similar to those used in tuna research and culture operations around the world. Its low cost, large internal volume (11,128.5 m^3), and its resiliency to dynamic and often damaging effects of the offshore environment make this enclosure the most appropriate type for observing the behavior of large pelagic fish in captivity. The enclosure proved to be very resilient to the damaging effects of a close pass of a hurricane and a tropical storm. A white, one-inch, straight-hung mesh net constituted the vertical walls and bottom of the enclosure.

The enclosure was anchored 32.2 km offshore of Wachapregue, VA, on the southwest corner of 20 Mile Hill—a bathymetric feature that rises within 33.5 m of the ocean surface in deeper surrounding waters. This location is relatively near shore and close to a temporally and spatially reliable aggregation of small (~1 m) Atlantic bluefin tuna regularly targeted by recreational fishermen.

The vertical temperature profile (°C), dissolved oxygen (mg/L), pH, suspended solids (NTU), Secchi depth (m), and conductivity (ppt) were monitored twice daily inside and outside the enclosure at 3-m intervals to a minimum depth of 15 m.

A pattern of seven, single-hook trolling lures were fished from a 18.3-m commercial vessel on 13-kg or 22-kg class trolling gear to capture fifty bluefin tuna in the vicinity of the study enclosure in June and July 1996. Tuna were subdued as quickly as possible and landed in a specialized cloth stretcher. We recorded the fork length (cm), approximate weight (kg), and general condition of each fish and released the fish into a 2400-liter elliptical transport tank. Compressed, bottled oxygen was employed to elevate

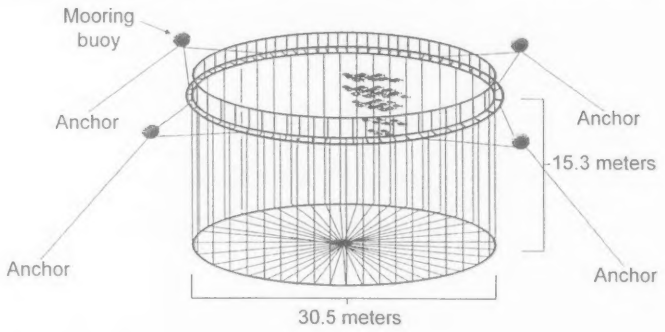

Figure 1

Illustration of the study enclosure. The tuna are scaled in relation to the enclosure. Spatial relationships are slightly distorted owing to the three-dimensional perspective of the illustration.

the levels of dissolved oxygen within the transport tank to ease the physiological stress associated with strenuous activity. Fresh seawater was continuously pumped into the tank to eliminate metabolic waste and maintain water quality. The tank had a padded top that minimized the potential for injury to fish during transport and reduced water loss resulting from boat movement. Viewing ports in the padded top allowed observation of the specimens while in transport. Physical contact with individual tuna was minimized, and when contact was necessary, only well-padded devices were employed. Transport time of individual fish was variable, but generally less than 3 hours. The number of fish simultaneously transported in the tank was controlled to avoid crowding and the depletion of dissolved oxygen. The tuna were recovered from the transport tank by using a cloth stretcher and released into the net-pen enclosure. Divers in the enclosure released the tuna individually, ensuring that they recovered proper spatial orientation upon release. Released specimens were assimilated quickly into the existing shoals of captive fish.

Video cameras (Hi8) in waterproof housings were used to record school structure of the captive tuna. A shutter speed of 1/2000 second was used to optimize the resolution of still frames within the constraint of available subsurface (<15.3 m depth) light. The automatic focus feature of the camera was disabled to avoid rapid fluctuations in focal depth from the intended subject to particulate matter suspended in the water column. Observations of schooling tuna were recorded with stand-alone cameras mounted inside the enclosure and with hand-held cameras during observation dives. Cameras mounted to the floating net pen on specialized polyvinyl chloride pipe structures were stabilized with elastic compensators to lessen movement caused by wave energy. Mounted cameras were positioned to provide head-on and subsequent perpendicular views in relation to the axis of motion of a school. This filming strategy allowed a more accurate observation of the characteristics of the school in three dimensions. Schooling was recorded more efficiently by using hand-held cameras during dives than through use of mounted cameras. During a dive, the entire internal volume of the enclosure was often visible from a given point, allowing a diver to anticipate the path of travel of a tuna school and to reposition the camera to attain the best possible images. Regardless of the apparent ease of tuna schools, divers positioned themselves against the enclosure's external wall to ensure minimal behavioral modification in the filmed schools. Video recordings from both mounted cameras and from held cameras were used in analysis.

Laboratory video analysis

Video recordings of ABT schools were reviewed, and 74 incidences of schooling in which an entire target school was visible were used for further analyses. All observations took place during daylight hours (0900 and 1600 h) and none took place during or within one hour of feeding events. An image analysis system that allowed digitization of points directly from a (paused) video source was employed for more precise quantification of school characteristics (Fig. 2). The system employed a video scan converter that overlaid the image output from a computer video source (640×480 pixel resolution) upon the video source image. The video scan converter allowed the user to select a color in the computer video overlay to be made transparent, revealing the underlying video signal (Fig.2). When employed in conjunction with an image analysis software package (SigmaScan Pro) on a personal computer, all the features of the image analysis software could be used on any still (paused) video source image without the use of a video frame-grabber. The position of each individual fish and school depth intervals were delineated by using the graphic capabilities and the Cartesian coordinate system of the image analysis software. The positions of individual fish in the school could be marked while the recording was advanced or reversed frame by frame, allowing the identification of poorly illuminated fish or fish that may have been hidden by individuals in the foreground of the school.

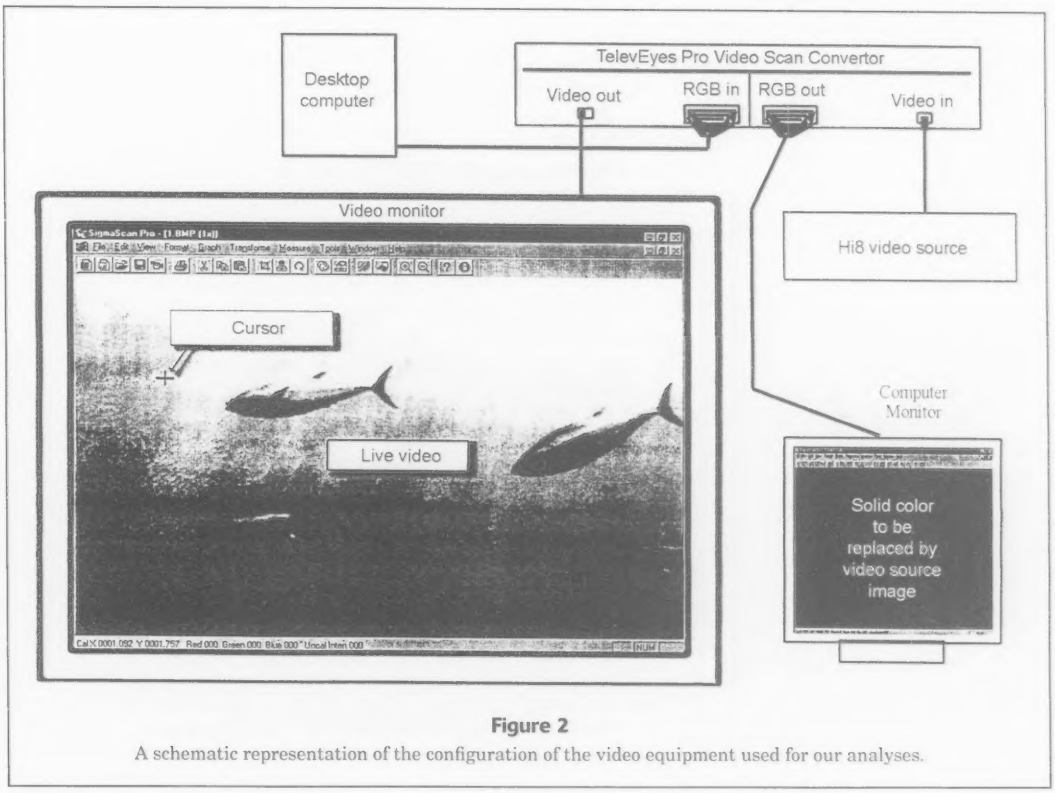

Figure 2

A schematic representation of the configuration of the video equipment used for our analyses.

Data analysis

School size Groups of tuna were considered to be schools and were included in the analyses if they were a polarized group (multiple individuals maintaining lateral proximity to neighbors and actively maintaining the same direction of travel during an observation period). The total number of fish in each school was counted and the frequency distribution of school size was determined. Because the observations were assumed to be independent and the total sample size was less than 2000, the normality of the distribution was tested by using the Shapiro-Wilks *W*-test. Least-squares regression was then used to evaluate the relationship between school size (N_s) and each environmental variable.

Predicting number of fish in school from surface counts Our video footage was filmed at an oblique perspective to the upper boundary of schools occurring within two meters of the water's surface. Measurement of school characteristics in body lengths or meters was not possible because of the camera angle or because of poor image resolution due to low light or high turbidity level. The total number of individual fish (N_s) was determined and the distribution of individuals within the school was described in terms of five depth intervals (Fig. 3). The surface interval included fish that were at the immediate surface of the school or fish that overlapped other fish at the surface on the horizontal plane. Each of the four subsequent intervals encompassed 25% of the remaining depth of the school. The number of fish per interval was designated as N_i ($_i$=the number of tuna in the i^{th} depth interval). Fish positioned at an interval boundary were assigned to the interval in which the greater portion of their body volume was positioned. Analysis of covariance (ANCOVA) (Sokal and Rohlf, 1995) was employed to detect differences in the slopes of each of the regressions of N_i on N_s in order to determine whether the distribution of individuals into school depth intervals changed in proportion to school size.

Three individual least-squares regression models were used to predict school size. The relationship between the number of individuals in the surface interval (N_1; independent variable) and the number of individuals in the remainder of the school (N_S; dependent variable) was first explored using simple least-squares regression (Sokal and Rohlf, 1995). The distribution of N_S given N_i was heteroscedastic necessitating the use of a weighted least-squares regression model by using a weight of 1/variance (Kleinbaum et al., 1988). A similar-weighted multiple-linear-regression relationship between N_1, N_2 (independent variables), and N_S (dependent variable) was developed because fish below the immediate surface of the school are sometimes seen and counted in photographs.

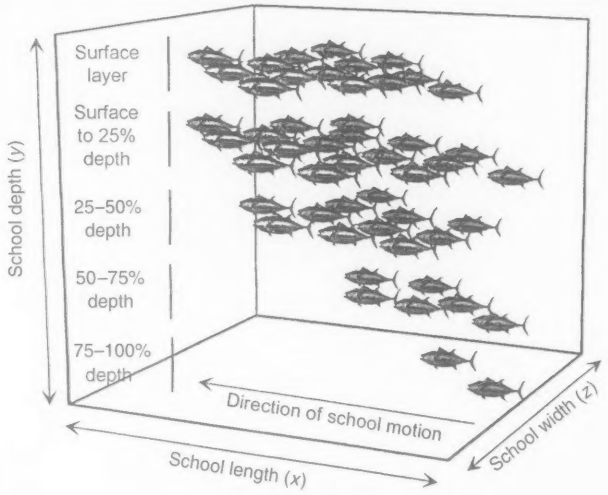

Figure 3

Representation of a school of 50 Atlantic bluefin tuna. Individuals have been separated into depth intervals according to the mean values for all school sizes. Layers are arbitrarily separated in the figure to allow visualization of school structure.

Predicting number of fish in school (NFS) from school dimensions School length, width, and depth in number of individual fish were recorded as indicators of school shape (Fig. 3). As in the prediction of NFS from surface counts, measurements were recorded in number of fish, not metric distance, because a precise spatial scale could not be established consistently. The lack of an accurate spatial scale also precluded the measurement of fine-scale school structure such as interindividual distances. School dimension measures were recorded according to the movement axis of a school. Length in number of fish was measured along the axis of school motion (x); depth was measured vertically (y) and width (z) was measured perpendicular to x on the horizontal plane (Fig. 3). School length and width were analyzed in relation to school size by least-squares regression. Regression models employed each one or both dimensions (i.e. length, width, length and width) of school shape as independent variables, and N_S as the dependent variable. Depth data were not analyzed in relation to N_S because these data may not be collected practically from wild schools. The relationship between dimensions of each school and selected environmental variables was examined by using least-squares regression.

Results

Behavior of the specimens

The captive specimens used in our investigation exhibited a high degree of awareness of the walls of the enclosure, even during periods of excited behavior. No collision with or brushing of the net wall was observed from above the surface or in the analysis of diurnal activities from underwater video footage, and no evidence of nocturnal collisions was observed. After a brief period of acclimation, the tuna did not actively avoid divers in the enclosure; they reacted only to avoid collision.

Visualizing the model

A three-dimensional model of the typical structure of a school of ABT was constructed based on the mean characteristics of the schools analyzed and on qualitative observations of school structure (Fig. 3). The proportionate distribution of individuals within school depth intervals varied little (see ANCOVA results below), suggesting that a single model adequately describes the mean vertical distribution of individuals for schools of varying size.

Number of schools and NFS

When a single school comprised the entire group of 50 tuna, less than approximately 20% of the enclosure volume was involved in containing such a school (senior author, pers. obs.). Fish swimming within such a school were observed to travel along a slowly arcing path around the entirety of the enclosure without making sharp turns. Smaller schools (up to 25 individuals) occupied only a very small portion of the volume of the enclosure. Single large schools separated into two or more smaller schools and joined back together with fluidity. When more than one school was observed simultaneously, each school exhibited movement independent of another. If two schools came close to one another in the enclosure, they would either pass by, move through the other group, or join together to

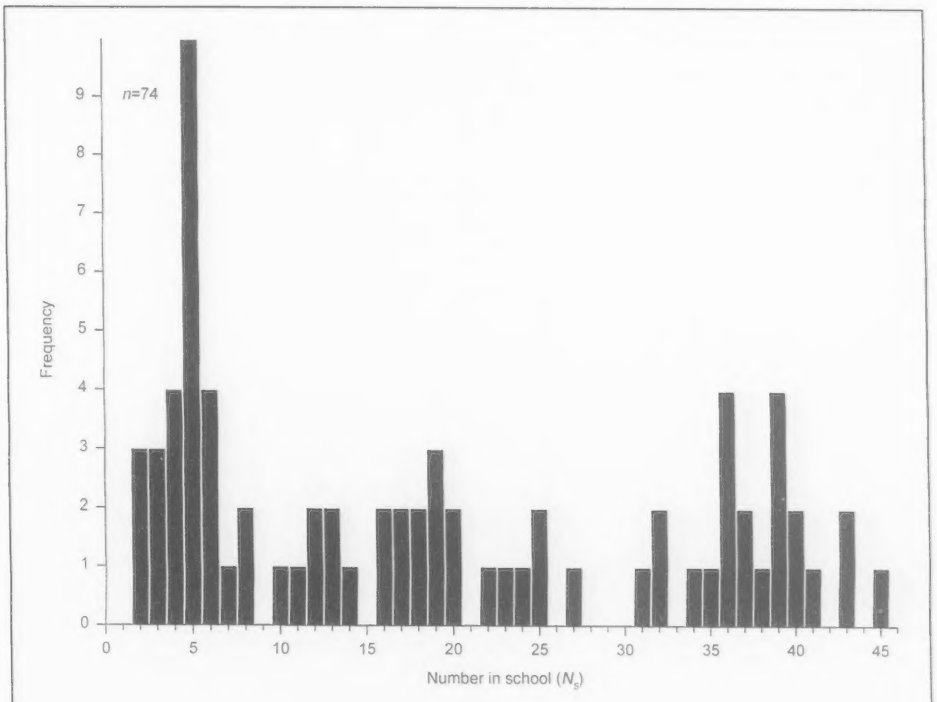

Figure 4
Frequency distribution of school sizes of Atlantic bluefine tuna.

form a larger group. While schools were remixing during such encounters, portions of one group would sometimes join another, maintaining the same number of independently acting groups, but changing the number of individuals within each group.

Particularly in larger schools, individual fish positions were observed to be dynamic, yet the overall shape of the school remained relatively constant. The mean size of schools observed was 18.88 individuals (n=74, SD=13.90). The smallest schools observed had 2 (n=3) individuals, and the largest had 45 (n=1). The frequency distribution of school sizes was not normal (Shapiro-Wilks' W, P<0.0001) and had two prominent modes centered at 5–10 individuals and 35–40 individuals (Fig. 4).

No statistically significant relationships between environmental variables and N_s were observed (Table 1). However, low power due to small sample sizes may have reduced our ability to detect significant effects.

Predicting NFS from surface counts

The relationship between the number of individuals in each depth interval and school size was linear in all cases. Although the r^2 values for each N_i–N_s regression were relatively low, their slopes appeared to be similar and were within a narrow range (0.14–0.23). However, ANCOVA revealed that the slopes were significantly different (P<0.001). Although the slopes were significantly different, the number of individuals in each interval remained in the same proportion except at low school sizes (<15 individuals).

The regression model incorporating the number of fish in the surface interval of the school as the independent variable and the number of individuals in the remaining portion of the school as the dependent variable had an r^2 of 0.67 (P<0.0001) (Eq. 1, Table 2). However, this regression model is likely biased owing to heteroscedasticity in the dependent variable. A second least-squares regression model, incorporating a weight of 1/variance, achieved an r^2 of 0.74 (P<0.0001) (Eq. 2, Table 2). The third regression model incorporated the number of fish in the surface interval (N_1) and the second interval (N_2) of the school as independent variables, the number of fish in the remaining portion of the school as the dependent variable, and 1/variance as the weight. This model had an r^2 of 0.70 (P<0.0001) (Eq. 3, Table 2). Partial F-tests for these models could not be executed because the dependent variable N_s–(N_i+ . . . N_i) changed depending on the number of school depth intervals used to predict NFS.

Three least-squares regression models were used to predict school size from school length and width. The model using length as the independent variable and N_s as the dependent variable had an r^2 of 0.74 (P<0.0001) (Eq. 4, Table 2). The second model predicted N_s from school width, achiev-

Table 1

Mean, minimum, maximum, and standard deviation of environmental data (pH; °C=degrees Celsius; mg/L=milligrams/liter; ntu=turbidity units; ppt=parts per thousand) recorded during schooling observation periods. The r^2 and P-values for linear regressions of each school measure on each environmental variable are provided. Measurements of dissolved oxygen were discontinued due to instrument failure.

Date	Observation start time	pH	Temp. (°C)	Dissolved oxygen (mg/L)	Total suspended solids (ntu)	Conductivity (ppt)
27 Jun 1996	10:00 AM	—	—	—	—	—
21 Jul 1996	3:30 PM	8.51	22.90	9.37	—	27.45
6 Aug 1996	10:11 AM	8.42	22.18	8.62	0.12	28.65
7 Aug 1996	8:45 AM	8.43	24.18	8.39	0.23	28.98
15 Aug 1996	3:45 PM	8.44	23.84	—	0.60	28.06
17 Aug 1996	10:48 AM	8.46	23.86	—	0.77	28.42
22 Aug 1996	11:55 AM	8.47	23.40	—	0.05	27.63
23 Aug 1996	11:15 AM	8.50	23.50	—	0.45	29.63
Mean		8.46	23.41	8.79	0.37	28.40
Minimum		8.42	22.18	8.39	0.05	27.45
Maximum		8.51	24.18	9.37	0.77	29.63
Standard deviation		0.04	0.68	0.52	0.28	0.76
Coefficient of determination	N_s	0.1*	0.15*	0.84*	0.09*	0.04*
	School length	0.01*	0.19*	0.91*	0.02*	0.01*
	School width	0.09*	0.22*	0.46*	0.1*	0.03*

* P>0.10.

Table 2

Regression equations employed to predict school size. The results of partial F-tests indicated that model 6 is the best predictor of school size (N_s). N_1, N_2, school length, and school width were measured in individual fish.

Model	Equation	n	r^2	partial F
1. N_1 vs. N_s	$N_s = (0.0337867 + 3.3173368 \times N_1) + N_1$	74	0.67*	
2. N_1 vs. N_s (weight=1/variance)	$N_s = (0.2042865 + 3.1849359 \times N_1) + N_1$	74	0.74*	
3. N_1, N_2 vs. N_s (weight=1/variance)	$N_s = (-1.412557 + 1.8516536 \times N_1 + 0.4190831 \times N2) + N_1 + N_2$	74	0.70*	
4. *Length* vs. N_s	$N_s = -6.769737 + 5.5334126 \times length$	74	0.74*	
5. *Width* vs. N_s	$N_s = -6.70494 + 5.7894971 \times width$	74	0.79*	
6. *Length, width* vs. N_s	$N_s = -9.788046 + 3.6463236 \times width + 2.7083604 \times length$	74	0.86*	(6 vs. 4) * (6 vs. 5) *

* P<0.0001.

ing an r^2 of 0.79 (P<0.0001) (Eq. 5, Table 2). The final, multiple regression model used school length and width to predict N_s (Eq. 6, Table 2, Fig. 5) with an r^2 of 0.86 (P<0.0001). Partial F-tests revealed that the final multiple regression model explained significantly more of the variation in school size than either length or width separately. No significant relationships were seen when school dimensions were considered in terms of environmental factors (Table 1).

Discussion

School structure

The non-normal frequency distribution of NFS and the appearance of modes in our data (Fig. 4) suggest the formation of elective groups of particular number. Previous investigations in school size show that fishes actively assemble into elective group sizes dependent upon the

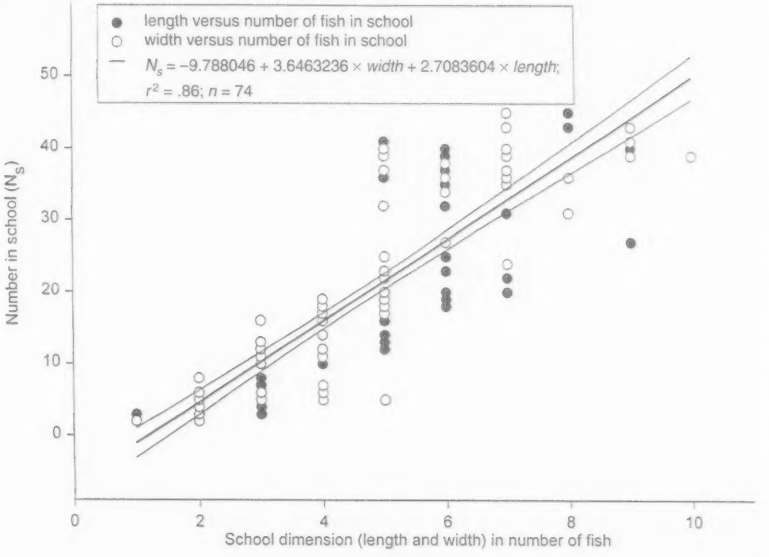

Figure 5

School size predictions based on multiple linear regression of total school number (N_s) on school length (L) and width (W).

interaction of predation risk, food availability, migratory status, and the species' life history (Hager and Helfman, 1991; Pitcher and Parrish, 1993). Elective group size for a species differs continuously under natural conditions with varying abiotic and biotic factors. At a discrete point in time when two schools were present in the enclosure, the size of one group determined the maximum number of individuals that could be in the other. However, there were no barriers to formation of groups numbering anywhere from 2 to 50 individuals. Therefore, the modes in group number that we observed should have resulted from underlying behavioral tendencies rather than enclosure-induced limitation of group size. If elective group sizes form in response to environmental conditions, then the range of environmental conditions during the study period was probably insufficient to detect environmental influences on NFS, and therefore elective group sizes.

The range of school sizes that we observed in the enclosure was limited by the number of individuals available to join schools. Photographs of ABT in the northwest Atlantic Ocean over a 50-day period in 1993 revealed that surface school counts ranged from 5 to 1294 individuals, and that the median school size was 84 individuals (Lutcavage and Kraus, 1995). The median value is well in excess of the maximum number of fish observed in the surface layer of our schools, emphasizing the importance of verifying the accuracy of our model predictions for larger schools with field data. When very large schools occur in relatively shallow water, the vertical depth of the school would be confined by the maximum water depth. A similar effect could be imposed by physical and chemical barriers such as vertical stratification in temperature and dissolved oxygen. Tagging studies may reveal more of the individual and group behaviors of this species in relation to the environment and assist in further understanding the way in which school structure may be affected by the physical and chemical environment.

Our results show that the vertical distribution of fish (in intervals) varies little across the range of observed school sizes. The ANCOVA of N_i on N_s illustrated that the slope of each regression was significantly different from all others (all $P<0.001$), but the biological importance of this difference is questionable because the slopes varied by less than 10%. Although the rate at which individuals are added to each interval varies as school size increases, there is no consistent trend in slopes among intervals. Furthermore, the statistical significance of the difference in slopes may be driven by the small standard error for each regression and extremely high power (>0.99).

The shape of large schools of bluefin tuna was less variable than that of small schools. Schools of less than 15 individuals are less vertically expansive, and generally one to three fish deep; larger (>15 individuals) schools are more than three individuals deep (Fig. 6). The pattern of increasing vertical depth continues to the largest school sizes observed, which are nearly always more than five individuals deep. The weakly cone-shaped profile of the model school depicted in Figure 3 is representative of the shape of most schools with more than 3 intervals. Smaller schools tend to be distributed in a vertically shallow, loosely oval profile. Our findings related to school structure are consistent with the observations of other investigators who have

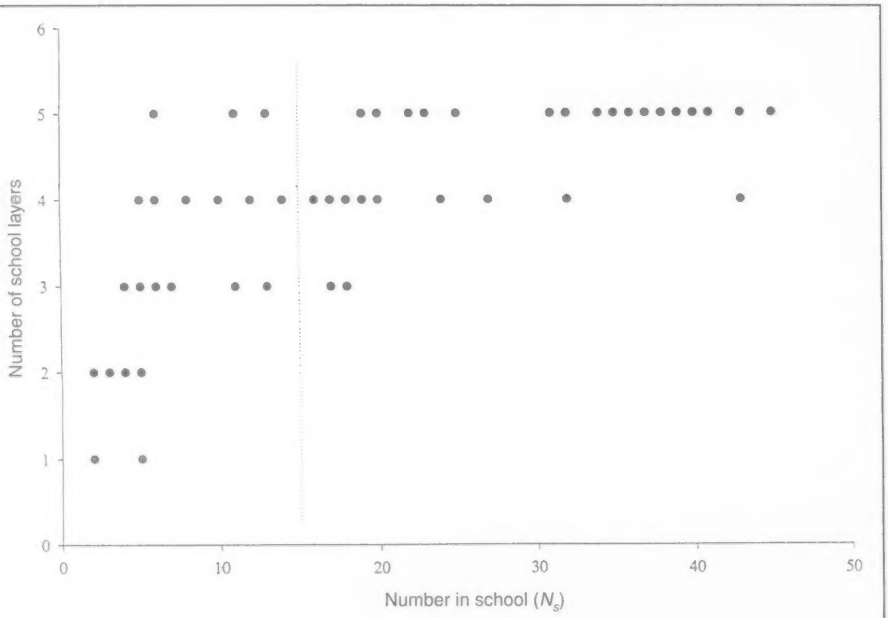

Figure 6

School depth in number of individuals plotted against total school number (N_s). The dotted line is drawn to illustrate the increase in the vertical expanse of schools coincident with the maximum number of individuals observed in parabola and echelon-shaped schools. The maximum number of depth intervals in our analysis was 5. Schools greater than 5 individuals in total depth are described by a surface layer and four 25% depth intervals.

observed that small schools have a strong horizontal aspect, and little vertical expanse. For example, Partridge et al. (1983) and Lutcavage and Kraus (1995) observed that small (<15 individuals) parabola- and echelon-shaped schools of noncaptive giant ABT vary little in shape. Interestingly, parabola and echelon shapes are not observed for large (>15) ABT schools, coinciding with changes in interindividual orientation between groups of less than 10 and 10–20 individuals (Partridge et al., 1983). This difference is similar to the shift in the depth of schools that we observed with approximately 15 individuals (Fig. 6). It is possible that Partridge et al. (1983) and Lutcavage and Kraus (1995) observed small schools in these configurations because larger schools expand vertically and adopt the semiconical shape that we describe. These results suggest that there may be a critical minimum number of individuals that must be present in a horizontal layer before schools begin to expand vertically, which would explain the limited size of two-dimensional ABT schools.

It is possible that ontogenetic variation in school structure exists, but understanding how such changes occur is critical in determining how variability in school structure could affect our modeling approach. Because our models use numerical relationships rather than distance metrics to predict school size, neither ontogenetic shifts in interindividual spacing or packing density changes related to school size should substantially affect the predictive ability of our models. However, if ABT schooling behaviors change at a more basic level due to enclosure or changing fish size, then our estimation techniques may be invalid outside captivity or with larger fish. Basic changes, such as vertical distribution of individuals within schools, the three-dimensional shape of schools, and strong school structure responses in relation to environmental factors, could all have serious effects on our modeling approach. Further, we feel that schools similar in form to those described as "densely-packed domes" by Lutcavage and Kraus (1995) could be described well by our models, but that numerical estimation of other school types might require the application of a different estimation technique.

Environmental effects on school formation

The physical environment may play a role in determining the vertical position of tuna in the water column (Holland et al., 1990; Block et al., 1997) or school structure (Partridge et al., 1983; Lutcavage and Kraus, 1995). No relationship between environmental variables and the shape of schools (quantitatively determined) or the vertical position of fish (in qualitative observations) were observed in our study, perhaps because of the small range and lack of vertical structure in salinity, pH, and dissolved oxygen

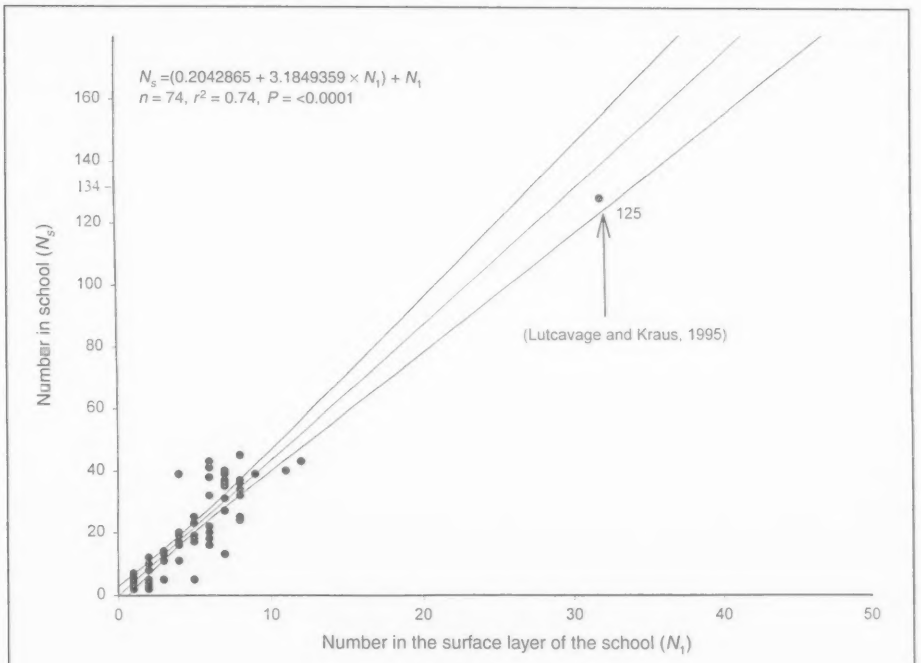

Figure 7

Comparison of school size observations to values predicted by the regression model using number of fish in the surface layer (N_1) weighted by 1/variance to predict school size (N_s). 95% confidence intervals are displayed for reference. The single data point from Lutcavage and Kraus (1995) is included for reference, but was not included in the regression model. The dotted line indicates the predicted value of school size from our model for the numbers at the surface observed by Lutcavage and Kraus (1995).

measurements (Table 1), the time when school structure data were collected, or low statistical power from small sample sizes. However, on a very limited number of occasions, the thermocline became situated within the enclosure at approximately 10 m. Only a few individual fish traversed the thermal boundary, and such excursions were very brief. Entire schools were not observed to cross to the cold side of the thermal boundary. The thermal profile is an important factor in determining the vertical distribution of Pacific yellowfin (*Thunnus albacares*) and bigeye (*T. obesus*) tuna (Holland et al., 1990). It is reasonable to assume that the thermal profile is important in the vertical distribution of Atlantic bluefin tuna as well, and that this effect was not detected in our study because of the periodicity of observations, the limited variation of environmental conditions within the enclosure, or the spatial constraints imposed by the enclosure.

Predicting NFS from surface counts

Our least-squares regression model predicts the number of fish in ABT schools from the number of individuals at the surface of the school without attempting to describe the fine-scale structure within schools in terms of interindividual spacing and orientation. The number of fish in the surface interval alone accounts for 74% of the variation in school size of three-dimensional schools similar to a densely packed dome. Because the only school type that we observed was similar to the densely packed dome, the applicability of our model to three-dimensional schools of other configurations is questionable and may be determined only by studying ocean schools of other configurations. Furthermore, the application of our model to two-dimensional schools such as parabolas, echelons, and surface sheets is not appropriate or necessary given the apparent lack of a three-dimensional component in their structure.

The only data point available to verify our model predictions is from a school described as "dome-shaped" that was photographed and subsequently captured by purse-seine (Lutcavage and Kraus, 1995). Thirty-two fish were counted at the surface of this school from an aerial photograph, and 125 "large giant" ABT were subsequently captured by the purse-seine vessel. We applied our model that predicts NFS from the number at the surface of the school to this datum to produce a NFS prediction. The prediction with our model of 134 individuals differed by 7% from the purse-seine capture of 125 individual fish, and was well within the 95% confidence intervals (Fig. 7) of model pre-

dictions. When ABT schools are captured by purse seine, it is assumed that nearly the entire school is captured.[2] If the net intersects an edge of the school while it is being deployed, the entire school will change its direction of travel in unison resulting in either the entire school being encircled in the net and captured or in the entire school escaping into open water. As a result, the estimate of total NFS from this data point is likely to be an accurate count of the number of individuals in the school. It is encouraging that our model so closely estimated the NFS of large giants considering that it was constructed from data for age 2+ fish that are a fraction of the size of large giant bluefin tuna. The accuracy of our prediction indicates the potential for generality of ABT school structure across both tuna size and NFS. However, substantial verification of our models is necessary before they may be applied to abundance estimation.

Predicting NFS from school dimensions

An alternative to using N_1 and N_2 to predict N_s is to use maximum school length and width. Identifying the longest and widest axis of the surface of a school and counting the number of individuals along these axes may yield more accurate estimates of the total NFS. The maximum dimensions of bluefin schools (length, width) had greater power as predictors of N_S than N_1 and N_2 (model 3 versus models 4, 5, 6, Table 2). Moreover, a combination of length and width to predict N_s produced a more confident estimate of school size than models using either variable individually. Irregularity in length and width at small school sizes likely introduced variation that reduced the individual predictive power of these variables. Inclusion of both length and width in the model to predict N_s could allow accurate prediction of small schools that are elongate or wide.

The regression diagnostics for the model using school length and width to predict school size (model 6, Table 2) suggest that it is the more reliable model for estimating NFS with aerial photographs. For the single open-ocean estimate available in the literature (Lutcavage and Kraus, 1995), the length and width of the school in number of individuals could not be determined. Model 6 may have the potential to yield a more accurate estimate of school size with a wider range of photograph qualities (as affected by sea state, water clarity, sunlight, etc.) because of its ability to predict NFS from partial surface counts. However, the utility of school length and width to predict N_s will remain uncertain until field data are available for thorough evaluation.

Enclosure effects

The effects of capture and captivity on school structure and behavior were points of concern in our study. Evaluating the effects that the enclosure had on school structure is problematic because the same factors that led to the use of an enclosure to make the observations preclude a direct *in situ* comparison. Because tuna are highly mobile and unpredictable in their movements, it would be difficult to obtain a number of school observations comparable to that collected from the captive fish in our study. Furthermore, the ability to approach schools in nature without significantly disturbing them is questionable, and effective observation at a distance that would not cause disturbance would be unlikely because of turbidity and the normal movement of schools. In this respect, a group of tuna that has become comfortable with the presence of human observers may be a better source of accurate school structure observations than a school of noncaptive fish that may perceive a human or mechanical presence as a predatory threat and react accordingly. Evidence of the acclimation of the study specimens to the enclosure was seen in their active and aggressive feeding behavior (Hanrahan and Juanes[3]) that was similar to the available anecdotal accounts of their open-water feeding behavior. The relation between fish length and enclosure dimensions may cause the impression that school formation was constrained heavily by captivity and that multiple schools could not achieve meaningful separation from one another (Fig. 1 illustrates a school of tuna at a scale of 1:1 to the enclosure). However, the relatively low density of fish in the enclosure (0.05 kg/m^3) allowed individuals to move in an uninhibited manner within the enclosure. Although the extent of enclosure-induced behavioral modification cannot be quantified in our study, the ability of our simple linear model to predict accurately the NFS for a noncaptive school is very encouraging.

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Effects of gear selectivity and different calculation methods on estimating growth parameters of bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae), from southern Brazil

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Abstract–We examined the effects of gear selectivity and the consequences of a chosen method for estimating growth parameters of bluefish, *Pomatomus saltatrix*, from southern Brazil. Samples were obtained from commercial landings at Rio Grande (1992–97). Age determination for 1159 fish indicated that gill-net and purse-seine fisheries caught 1–5 year-old and 1–7 year-old fish, respectively. Trawlers caught fish from ages 1 to 7. Because of their high degree of selectivity, the gill nets caught the larger individuals of ages 1 and 2, as well as the smaller individuals of ages 3–5. Faster-growing fish captured with gill nets had smaller scales than fish of a similar length caught with purse seines. By contrast, the slower-growing fish captured with gill nets had larger scale size than fish of a similar length caught with purse seines. For gill nets and purse seines, there were differences between the von Bertalanffy growth estimates derived from both mean values of back-calculated length-at-age and individual back-calculated length-at-age. We also recorded differences in growth parameters obtained from back-calculated length-at-age derived from measurements to the last annulus only and from measurements of all annuli up to the sampling age. Selectivity-related bias was incorporated in the estimation of growth parameters, yielding unrealistic estimates of L_∞ and k for the gill-net growth curve.

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The bluefish, *Pomatomus saltatrix* (L.) (Pomatomidae), is a highly mobile pelagic predator (Haimovici and Krug, 1996), widely distributed along the continental shelf in the temperate and warm waters of the Atlantic, Pacific, and Indian oceans (Wilk, 1977). Abundant in Brazilian waters, this species is commercially exploited by purse-seine and gill-net fleets operating in the subtropical coastal waters of Rio Grande do Sul between Conceição (31°42′S) and Chuí (33°43′S), at depths between 8 and 100 m (Lucena and Reis, 1998) (Fig. 1). Purse-seine boats are approximately 23 m in length and have 220–330 HP engines, whereas gill-net boats are usually 14–16 m in length and have 90–150 HP engines (Boffo and Reis, 1997). Gill nets used in the bluefish fishery are drift gill nets 1800 m in length and that have a stretched mesh size of 90 mm.

Commercial catches of bluefish were first monitored from 1976 to 1983 on the fishing grounds from Sarita (32°37′S) to Conceição at depths up to 35 m (Krug and Haimovici, 1991). Bluefish were then primarily exploited by purse seine but were also taken as bycatch in a gill-net fishery targeting wreckfish (*Polyprion americanus*) and elasmobranchs. Commercial catches were dominated by fish between 1 and 3 years old for the purse-seine fleet and by fish aged 2–4 years old for the gill-net fleet (Krug and Haimovici, 1989).

As catches from the estuarine Patos Lagoon declined, beginning in 1982, the artisanal fleet began exploiting new fishing grounds and a fishery developed in shallow coastal waters (Reis, 1992). Since 1990, bluefish have been caught primarily by gill net (up to 72% of landings by weight) and the fishing grounds now extend southward into waters up to 100 m (Lucena and Reis, 1998). For the period 1991–95, commercial catches have included individuals to age 10 (Lucena, 1997).

Since the emergence of the gill-net fleet, the exploitation of the bluefish has been increasing. From 1991 to 1996, there has been an 87% increase in the length of netting deployed and a 166% increase in the soak time of the gear (Lucena and Reis, 1998). In addition, there has been a decrease in mean length of fish caught by purse seines and gill nets from 1992 to 1995 (Lucena, 1997). Average annual landings of the bluefish decreased from 3100 (during the period 1990–95) to 1700 tonnes (during 1996–98) (IBAMA[1]).

The growth parameters of the southern Brazilian bluefish population were estimated by Krug and Haimovici (1989), using scales from fish landed commercially by several gears (gill nets, purse seines, trawlers, long lines, and beach netting) from 1976 to 1983. Their study excluded individuals larger than 63 cm TL because the larger fish lived

[1] IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renovaveis). 1990–1998. Unpubl. data. Centro de Pesquisa do Rio Grande (CEPERG)–RS, IBAMA, Brazil. Cep: 96201-900.

at depths greater than 35 m, the operational limit of the fleet at that time. Given today's increased exploitation of bluefish, and the lack of older fish in the earlier study, we decided to examine the age structure and growth parameters of the bluefish stock off southern Brazil. New data are compared to previously published information and the effects of gear selectivity on the estimation of growth parameters are also evaluated.

Annulus formation occurs in winter (June to September) and is associated with lower water temperature (Haimovici and Krug, 1996). Rings do not become visible until growth resumes in January or February. During peak spawning, January–February, the true age of individuals with one ring is 7 months on average (Haimovici and Krug, 1992).

Even though most sampled bluefish have usually been caught during the short period of the fishing season (June to September), the real age of a fish may vary by between 4 and 9 months from that notionally adopted because the bluefish is a multiple spawner and because this species' scale ring formation is associated with lower temperatures (the exact timing of ring formation varies from year to year and can also be influenced by environmental phenomena such as El Niño events). Hence, because sample collections were spaced unevenly throughout the fishing season for both within-year and between-period comparisons, growth rates based on changes in observed lengths were deemed inappropriate and the estimation of growth parameters was based on back-calculated lengths.

Many early writers, using the back-calculation technique, made no mention of the reason for choosing a particular procedure (e.g. Johnson and Saloman, 1984; Barger, 1990; Vieira and Haimovici, 1993). Valuable reviews on back-calculation were carried out by Hile (1970), Tesch (1971), Casselman (1987) and Francis (1990) and, although this technique is widespread, it still does not appear to be well understood. Moreover, there is a common tendency to plot only mean, rather than individual, back-calculated length-at-age to produce the growth curve (Hilborn and Walters, 1992). The effects of different approaches on growth parameter estimates are discussed in this paper.

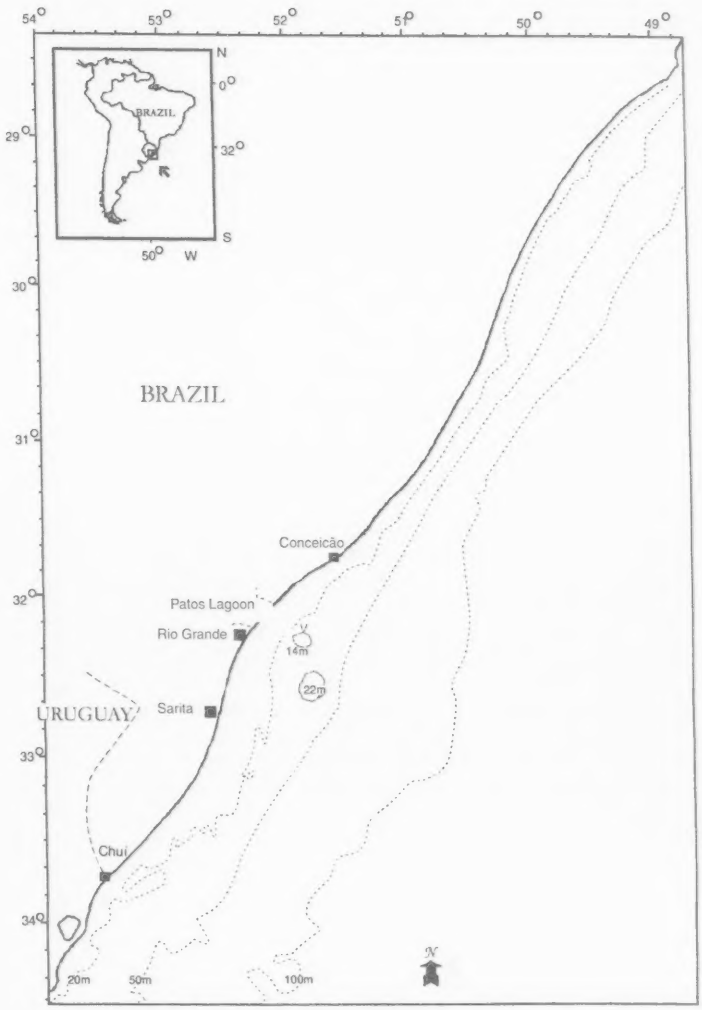

Figure 1

Map of the southern coast of Brazil and sampling area for bluefish.

Materials and methods

Sampling, age structure, and scale reading

We sampled commercial landings from three fleets at Rio Grande from 1992 to 1997: catch from the gill-net fleet, catch from the purse-seine fleet, and bycatch from the trawl (pair and otter) fleet (Table 1). We sampled 13 additional bluefish from the gill-net fishery targeting wreckfish (*Polyprion americanus*), choosing larger (older) fish in order to investigate the size of the bluefish that move along the shelf break at depths >200 m, where the wreckfish fishery operates. We collected an additional 57 individuals (75–135 mm in TL) with experimental trawls in the estuary of Patos Lagoon in order to calibrate the weight-length relationship for juveniles.

Total length (TL) in mm of 1159 fish (260 to 711 in TL), measured to the end of the extended tail were recorded. The total weight (TW, in grams) and sex (macroscopically determined) of 580 bluefish, ranging in size from 75 to 711 mm TL, were determined to calculate the weight-length relationship, by using nonlinear least-squares regression

Table 1

Depth, sampling period, length and age range, and number of boats and individuals examined as part of the bluefish sampling in southern Brazil during the 1992–97 period.

	Bluefish target species		Bluefish bycatch	
	Gill net	Purse seine	Gill net	Trawl (pair and otter) net
Depth (m)	10–35	8–100	270	8–68
Sampling period	Jun–Aug	May–Sep	Sep	Jan–Mar, May
Length range (mm)	301–602	265–711	650–770	262–711
Age range (years)	1–5	1–7	5–10	1–7
Number of boats examined	33	11	1	7
Number of individuals examined	853	233	13	60

(SPSS, 1998). *T*-tests were used to test for gear- and sex-specific length-weight relationships.

Scales of captured bluefish were removed from under the pectoral fin and stored in a coin envelop. The scales of 1159 fish were mounted between glass slides and fish age was determined by following Krug and Haimovici (1989). Fish were considered to be 1 year old after the formation of the first winter ring and a further year was added to the age of the fish for each subsequent ring (Krug and Haimovici, 1989).

Two different readers assigned ages for the fish, with no knowledge of fish length or month and gear of capture. If two readings agreed, then the age of that reading was adopted as definitive. If not, the scale was reread after two months, concurrently by both readers. If two of the three readings were the same, the age of these two readings was assumed as definitive. If all three readings differed, the scale was omitted from further analysis. On some scales, a single reader assigned ages. This method was followed with a time lag (2 months) between readings.

Estimation of growth parameters

Sample sizes were sufficient for estimation of growth parameters from gill-net and purse-seine samples. Ages from the direct reading of scales were obtained for 816 bluefish from gill-net samples and for 212 bluefish from purse-seine samples.

Ricker (1992) pointed out that the inclusion of sampled fish taken at different times during the growing season should be avoided and that preferably fish collected between the formation of an annulus and the start of the next year's growth should be used in analysis. Only data obtained during the fishing season were included for estimation of growth parameters derived from direct reading, and data derived from purse-seine samples (the less selective gear) were used to compare the growth parameters derived from mean observed lengths-at-age and back-calculated lengths-at-age.

For back-calculation of growth parameters, 283 specimens (174 from gill-net samples and 109 from purse-seine samples) were used. Fish were processed by sex and gears used in their capture to avoid bias in results.

We determined the relationship between fish length (TL) and scale radius (S). Krug and Haimovici (1989) previously identified a nonlinear relationship between fish length and scale radius for the southern Brazilian bluefish ($TL=aS^b$). Back-calculated total lengths at age were determined by using the formula of Monastyrsky (see Bagenal and Tesch, 1978; Francis, 1990):

$$L_n = (S_n / S)^b \times TL,$$

where L_n = the length of fish when annulus "n" was formed;
S_n = the radius of annulus "n" (at fish length L_n); and
b = the slope from the body-scale relationship TL on S.

This nonlinear method for back-calculation assumes that the relationship TL on S is of the form $TL = aS,^b$ where a and b are parameters to be estimated usually by nonlinear least squares.

The periodicity of growth increments was examined by using analysis of marginal increments $[(S - S_n)/(Sn - S_{n-1})]$ from fish 2–7 years old (mainly 2–3 years old) (following Krug and Haimovici, 1989). Because of the limited temporal range of samples, no marginal increments analysis was done for the months March–April and November–December.

We derived theoretical growth parameters by fitting back-calculated lengths-at-age to the von Bertalanffy (1934) growth equation

$$L_t = L_\infty\left(1-\exp^{-k(t-t_0)}\right),$$

where L_t = the length at age t;
L_∞ = the asymptotic length;
k = the brody growth coefficient; and
t_0 = the age when length would theoretically be zero.

Estimation of growth parameters was based upon two criteria. Criterion I identified the type of summary statistic to be used:

Ia corresponded to mean values of back-calculated lengths-at-age; and
Ib represented individual back-calculated lengths-at-age.

Criterion II identified the type of data to be used:

IIa corresponded to the back-calculated length-at-age derived from the last annulus only; and
IIb represented the back-calculated lengths-at-age derived from all the annuli up to the sampling age.

We calculated growth parameters by using four methods for each gear: method 1 (where criteria Ia and IIa were used), method 2 (where criteria Ia and IIb were used), method 3 (where criteria Ib and IIa were used), and method 4 (where criteria Ib and IIb were used).

The growth curves were estimated with the program AD Model Builder (Otter Research, 1996), which uses an automatic differentiation algorithm to estimate the parameters of a nonlinear function with an appropriate objective function. The software allows the calculation of confidence intervals for parameter estimates with asymptotic normal approximations.

The objective function considered is the nonlinear least-squares regression criterion, $R(.)$, either given by

$$R\left(L_\infty, k, t_0 | O_t\right) = \sum_{t=1}^{T} (O_t - L_t)^2,$$

when mean values are considered, or given by

$$R\left(L_\infty, k, t_0 | O_{ti}\right) = \sum_{t=1}^{T} \sum_{i=1}^{n_t} (O_{ti} - L_t)^2,$$

when individual values are considered.

Note that O_t is the mean back-calculated length-at-age t, O_{ti} is the ith individual back-calculated length-at-age t, T denotes the maximum number of distinct ages, and n_t is the number of observations at age t. To compare growth curves between gears, Hotelling's t-test (1979) was used.

Results

Age structure of the stock

We examined a total of 1159 fish and 94% of samples were collected during the fishing season (June to September)—the remaining numbers represented bycatch during the off-peak season. Age determination (Fig. 2) indicated that the gill-net fisheries caught fish of ages 1–5 (mode=3 yr) and purse-seine fisheries caught fish of ages 1–7 (mode=2–3 yr). Trawlers caught fish of ages 1–7. Individuals of ages 5 to 7 represented less than 2% of samples (individuals of 5 to 7 years old were underrepresented also in the back-calculation procedures). The deep-water (to 200 m) gill-net fishery targeting *P. americanus* caught individuals of age 5–10 and the only fish older than age 7. There was a considerable overlap in the range of observed lengths-at-age (Table 2), nevertheless significant differences between gears were detected for some ages. Gill nets caught larger individuals of ages 1 and 2 and smaller individuals of ages 3–5 than purse seines. Trawls, on the other hand, appear to catch smaller individuals in all age classes.

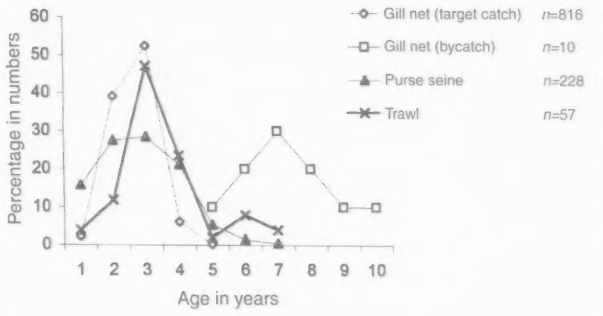

Figure 2

Relative proportion (%) of bluefish caught by various fisheries operating off Brazil's southern coast.

Weight-length relationship

We found no statistically significant differences in length-weight relationships between gear types or between sexes (t-test, $P>0.05$). The relationship for both sexes and all gears combined is illustrated in Figure 3.

Body-scale relationship

Gear-specific regressions of TL on S (Fig. 4) showed that gill nets tended to catch the fast-growing fish of younger ages and the slow-growing individuals of older ages. These faster-growing younger fish (<360 mm TL, 1–2 years) have smaller scales than similar-size purse-seine–caught fish (t-test; $P<0.05$). By contrast, the slower-growing fish (>410 mm TL, 4–5 yr) had larger scales than fish of a similar length caught by the purse-seine fleet. For the length range that the gill nets target most efficiently (360–410 mm TL, age 3), the TL-S relationship for both gill net and purse seine was similar.

Periodicity of growth increments

Our limited data showed marginal increment to be lowest in January–February, corresponding to ring deposition.

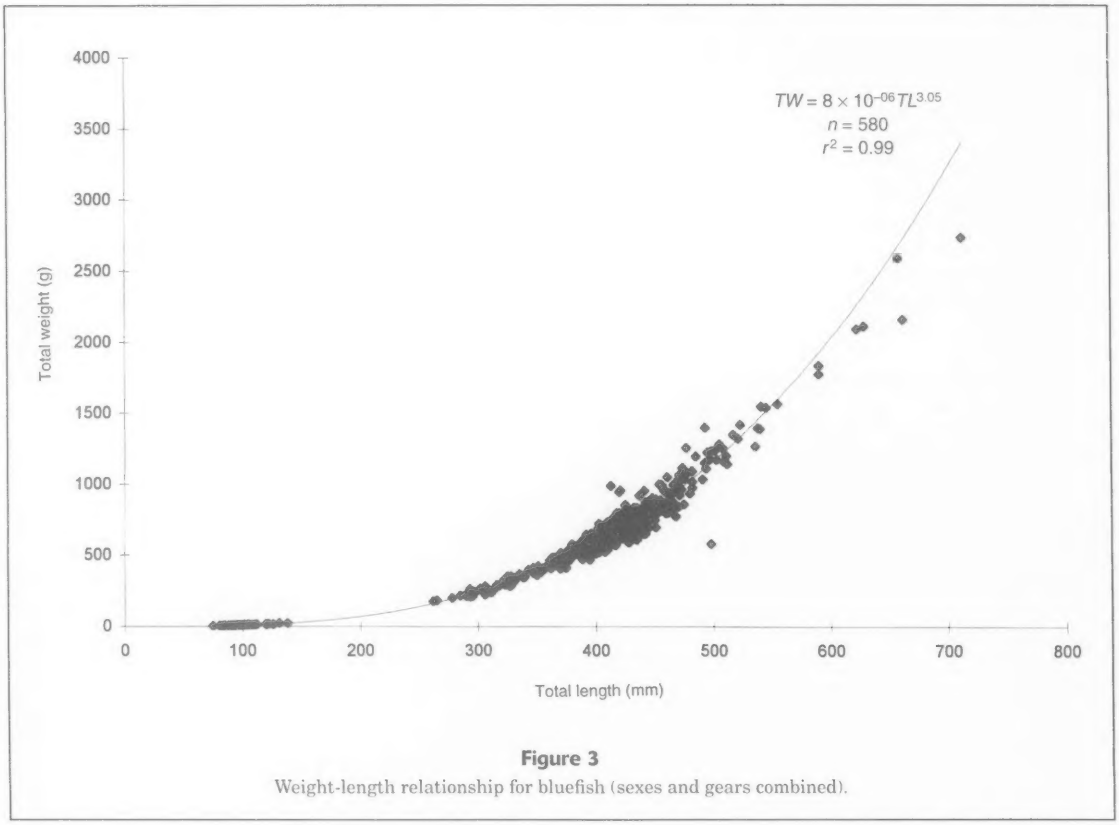

Figure 3

Weight-length relationship for bluefish (sexes and gears combined).

Table 2

Mean observed length-at-age for gill-net (targeted catch), purse-seine, and trawl catches. The symbol SD denotes the standard deviation, n denotes the number of individuals examined and "—" denotes that a value could not be calculated. ANOVA was performed only for the age classes with n greater than 10.

Age (yr)	Gill net length at age (mm)			Purse-seine length at age (mm)			Trawl length at age (mm)		
	Average	Range	SD (n)	Average	Range	SD (n)	Average	Range	SD (n)
1	354.3[1,2]	301–417	30.8 (33)	313.5[1]	276–385	23.4 (31)	278.5	265–292	19.0 (2)
2	408.7[1]	293–495	32.6 (286)	385.0[1]	290–485	44.5 (49)	372.8	285–409	47.0 (6)
3	438.9[1]	328–543	31.0 (431)	460.4[1,3]	364–520	40.7 (61)	419.4[3]	361–498	30.6 (24)
4	467.7[1]	410–602	42.3 (65)	518.1[1,3]	451–622	38.3 (62)	451.5[3]	342–554	54.7 (12)
5	498.0		— (1)	552.5	418–711	54.4 (17)	474	—	— (1)
6				622.7	556–661	38.7 (7)	507	477–661	89.3 (4)
7				664.5	657–672	10.6 (2)	651	642–660	12.72 (2)

[1] Significant difference between lengths-at-age for gill-net and purse-seine catches (ANOVA, $P<0.05$).
[2] Significant difference between lengths-at-age for gill-net and trawl catches (ANOVA, $P<0.05$).
[3] Significant differences between lengths-at-age for purse-seine and trawl catches (ANOVA, $P<0.05$).

Table 3

Mean back-calculated total lengths (mm) of bluefish for the purse-seine and gill-net fishery. *Lc* = Mean observed lengths-at-age. Mean back-calculated lengths-at-age derived from the last annulus are shown in bold.

				Method						
Age-group	*Lc*	*n*	SD (last annulus only)	1	2	3	4	5	6	7
Purse-seine fishery										
1	313.5	22	27	**202.2**						
2	385.0	26	31	189.0	**315.5**					
3	460.4	23	36	227.9	358.0	**437.3**				
4	518.1	26	35	234.5	373.1	462.4	**504.3**			
5	552.5	8	72	225.4	366.9	455.4	504.5	**547.1**		
6	622.7	3	30	219.1	352.5	447.2	502.0	552.7	**607.9**	
7	664.5	1	—	247.9	352.6	512.1	574.6	615.9	636.5	**647.4**
Mean (all annuli)		109		214.6	350.6	451.3	506.1	554.4	615.4	647.4
SD				36	47	45	46	64	28	—
Gill-net fishery										
1	354.3	18	27	**236.2**						
2	408.7	59	46	230.4	**346.1**					
3	438.9	75	30	239.8	358.2	**430.3**				
4	467.7	21	41	244.9	366.2	445.5	**480.8**			
5	498.0	1	—	196.3	350.3	403.7	426.5	**460.0**		
Mean (all annuli)		174		236.7	354.9	433.3	483.4	460.0		
SD				41	39	58	41	—		

Growth occurs from May to October and peaks in August. From these data, we believe that the growth increments are annuli.

Back-calculated length-at-age and estimation of growth parameters

Back-calculated lengths-at-age calculated by using the last annulus only (IIa) were smaller than lengths-at-age calculated by using all annuli (IIb) (Table 3). There were also significant differences in lengths-at-age for these two criteria between gear types (t-test, $P<0.05$ for all age classes). Sexes were combined because there was no significant difference between them (t-test, $P<0.05$). Back-calculated lengths-at-age for gill-net samples showed a decreasing pattern for older fish (5 years old), indicating the presence of Lee's phenomenon and the selective effects of this gear.

Estimates of theoretical growth parameters indicated differences by gear and method of estimation (Table 4, Fig. 5). For criterion I, estimates with mean back-calculated lengths-at-age (criterion Ia) produced higher values of L_∞ (and smaller values of k) for purse-seine samples than estimates with individual back-calculated lengths-at-age (criterion Ib). The opposite trend was observed for the gill-net samples.

On the other hand, for criterion II, especially for purse-seine samples, estimates of mean back-calculated lengths-at-age derived from the last annulus only (criterion IIa) produced smaller values of L_∞ (and higher values of k) than estimates with mean back-calculated lengths-at-age for all annuli (criterion IIb) (Table 4). Furthermore, for the purse seine, the growth parameters derived from methods 3 (Ib, IIa) and 4 (Ib, IIb) were distinct (Table 4). The at-

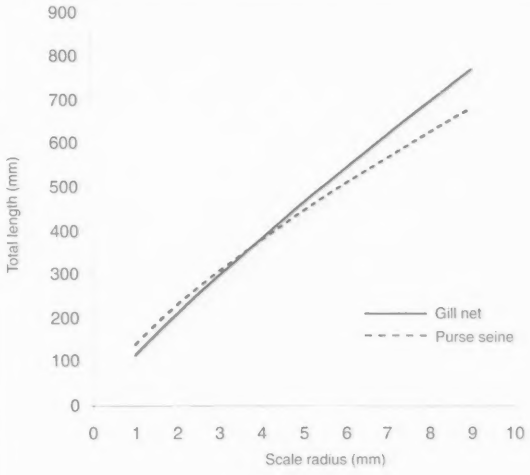

Figure 4

Total length-scale relationship derived from gill-net samples ($TL=140.5S^{0.72}$, $n=174$) and purse-seine samples ($TL=116.7S^{0.86}$, $n=109$).

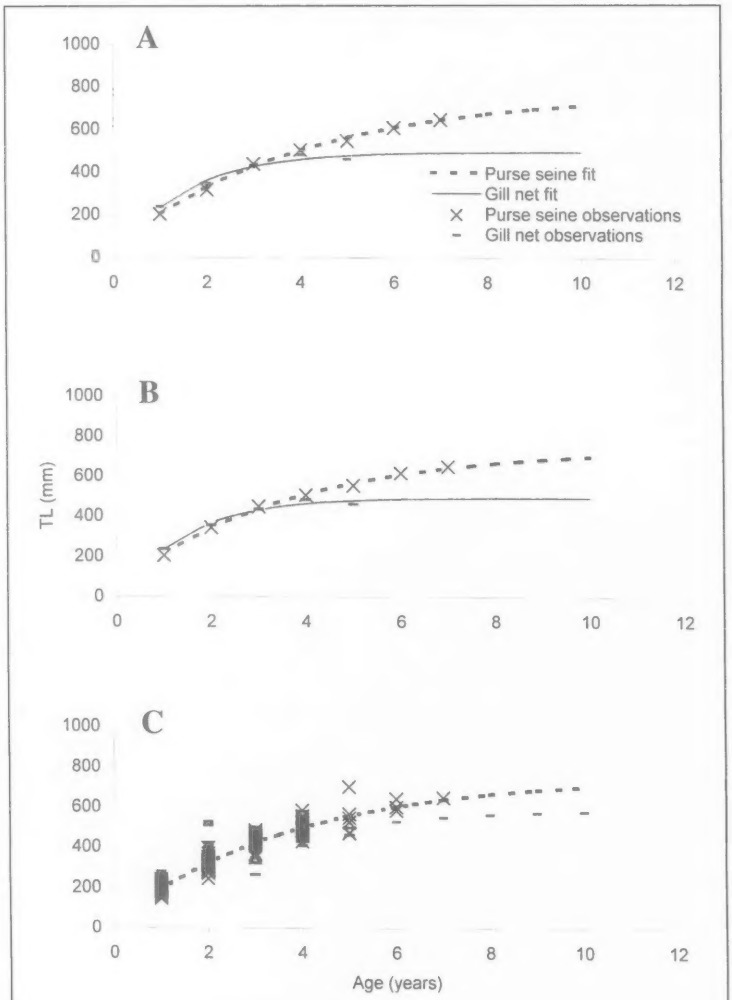

Figure 5

Growth curves for bluefish caught by purse seines and gill nets calculated by (**A**) method 1 (mean lengths—last annulus only) (**B**) method 2 (mean lengths—all annuli) and (**C**) method 3 (individual lengths—last annulus only).

tempt to fit the von Bertalanffy growth curve by method 4 for the gill net failed because no feasible solution could be found for the parameters of the curve.

For all methods there were significant differences in growth parameter estimates between the gill-net and the purse-seine samples (Hotelling's t-test, $P<0.05$).

The comparison between mean back-calculated (especially criterion IIa) and mean observed length-at-age showed that the latter is systematically larger than the former (Table 3). This difference was expected because the mean back-calculated lengths-at-age were obtained for length at time of annulus formation (where spawning season and time of annulus formation are considered uniform for all fish). The differences between both means may be attributed to growth after the growth mark formation. Moreover, the mean observed length-at-age calculated from sampled fish was obtained from individuals that may have spawned at different times of the year or whose ring formation may have been distinctly visible in time, resulting in fish with distinct real ages. Hence, growth parameters obtained from mean observed lengths-at-age of purse-seine catches during the fishing season (L_∞=985 mm, k=0.12 and t_0= 2.17) were significantly different from the growth parameters derived from back-calculated lengths-at-age (also from purse-seine catches) (Hotelling's t-test, $P<0.001$ for all methods).

Table 4

Growth parameters and correlation between k and L_∞ for different methods (1–4), and respective criteria (Ia, Ib, IIa, IIb) of back-calculation. Standard errors are given in parenthesis. "—" denotes that parameter estimates did not converge to a solution.

	Purse seine				Gill net			
Parameters	[1] Ia–IIa	[2] Ia–IIb	[3] Ib–IIa	[4] Ib–IIb	[1] Ia–IIa	[2] Ia–IIb	[3] Ib–IIa	[4] Ib–IIb
L_∞	773 (32.6)	743 (26.6)	754 (53.8)	670 (55.2)	496 (21.3)	491 (17.1)	589 (45.3)	—
k	0.25 (0.03)	0.27 (0.03)	0.26 (0.04)	0.35 (0.07)	0.65 (0.14)	0.71 (0.13)	0.39 (0.08)	—
t_0	−0.21 (0.12)	−0.27 (0.12)	−0.15 (0.12)	−0.09 (0.13)	0.03 (0.20)	0.09 (0.17)	0.27 (0.2)	—
$r(k, L_\infty)$	−0.97	−0.97	−0.98	−0.98	−0.89	−0.87	−0.98	—

Discussion

Back-calculation of length is widely used to obtain growth curves, to estimate length-at-age of individuals that are rarely observed, to compare growth differences among populations or sexes of the same species, and even to illustrate gear selectivity (Francis, 1990). However, this technique is poorly understood (Francis, 1990; Rijnsdorp et al., 1990) and some of the sources of bias in using the technique are 1) inaccurate counts of annuli and incorrect estimation of time of formation of the growth mark; and 2) an erroneous choice of the mathematical function to describe the body-scale relationship. In respect to the first source of bias, our results corroborate the findings of Krug and Haimovici (1989). In respect to the body-scale relationship, the main concern of Francis (1990) was to decide whether the appropriate regression was TL on S (body proportional) or S on TL (scale proportional). The small difference between the back-calculation derived from the two approaches for the bluefish can be a minimum measure of precision of the back-calculation procedure (Francis, 1990). Campana (1990) and Wright et al. (1990) pointed out that slow-growing fish have larger bony structures (scales or otoliths) than fast-growing fish of the same size. For the bluefish body-scale relationship, we attribute this effect to gear selectivity. The fast-growing fish (ages 1 and 2 from the gill-net catches) tend to occur above the purse-seine curve and the slow-growing fish (ages 4 and 5 from the gill-net catches) tend to occur below the purse-seine curve in a TL-S relationship.

An additional source of bias may be introduced depending on whether mean back-calculated lengths-at-ages or individual lengths-at-age are used to fit the von Bertalanffy equation. The estimated growth parameters derived from the two methods may differ considerably (Hilborn and Walters, 1992). Use of mean values would ignore individual variability in length-at-age, giving the same weight for possibly uncertain ages because of low sample sizes.

We suggest that back-calculated lengths-at-age derived from the last annulus only (criterion IIa) be used rather than the back-calculated lengths-at-age derived from all annuli (criterion IIb). Many investigators continue to use mean-weighted data (all annuli) (e.g. Krug and Haimovici, 1989; Barger, 1990)—a procedure in violation of the least-squares assumption of independence of sample elements (Draper and Smith, 1966). This assumption is violated when multiple measures from a single fish are used. Use of the last annulus only does not use all information available about growth of all cohorts and may result in an incorrect estimate of lengths-at-age for age classes absent or infrequently caught. However, if representative samples are available for younger age groups, the use of back-calculated lengths-at-age derived from the last annulus only is to be preferred.

Ricker (1969) noted that where differential mortality exists, the calculated average length of a particular age class calculated from the last annulus differs from the calculated average length of an age class calculated from previous annuli—the latter representing the former size of the fish that has survived to the sampling age. The differential mortality may be due to natural or fishing causes but under these circumstances, the average size of fish in the year class becomes different as time passes and the frequency distribution of the survivors would become progressively skewed. Gutreuter (1987) suggested that back-calculation should be restricted to the most recent annulus to avoid bias from size-selective sampling.

Gear selectivity can influence estimates of growth parameters (Ricker, 1969; Potts et al., 1998), and we found that gear-related differences in parameters were significant. Gill nets, on the basis of their mesh size, are a selective gear. Trawls are selective for smaller fish that may not be able to avoid the nets because of slower swimming speed (Hilborn and Walters, 1992). Purse seines are probably a less selective gear (Cushing, 1968). Observed lengths-at-age reflect the differences in selectivity between gears. Gill nets catch the faster-growing fish at age 1 and 2 and the slower-growing fish at age 4 and 5, and trawls catch the smaller individuals for each age.

The high selectivity of gill nets is visible in the body-scale relationship, the mean observed length-at-age, and in the back-calculated lengths-at-age which indicate the presence of Lee's phenomenon (Lee, 1920). Size-selective mortality—caused by the differing catchabilities of fish at different sizes—is the probable reason for Lee's phenomenon in back-calculation of lengths from gill-net catches.

Table 5

Back-calculated length-at-age and growth parameters obtained by various authors and at a number of locations. Probability values calculated from tests between means ($P<0.05$) for the bluefish in southern Brazil (our study and published studies) are given in parentheses. Mean back-calculated length-at-age derived from all annuli was used to allow comparison between studies.

	Age class											
Location	1	2	3	4	5	6	7	8	9	k	L_∞	Source
Black Sea	212	322	409	562	615	663				0.150	1080	Kolarov (1963)
Senegal	209	375	500	604	689	758	913	858	893	0.018	1044	Champagnat (1983)
Long Island, USA	230	400	490	580	640	690	710			0.340	795	Richards (1976)[1,2]
Mexico	308	413	509	576	627	675	715	766		0.180	944	Barger(1990)[1]
West Atlantic	290	361	415	473						0.096	1019	Barger (1990)[1]
South Africa	165	279	369	437	480					0.196	840	Van de Elst (1976)
Southern Brazil	196	356	438	506	562	600	618			0.380	662	Krug and Haimovici (1989)
Southern Brazil	214	351	451	506	554	615	647			0.260	754	Our study
	(P=0.0)	(P=0.21)	(P=0.03)	(P=0.99)	(P=0.57)	(P=0.39)	—[3]					

[1] Fork length was considered.

[2] Growth parameters were calculated from published mean back-calculated length-at-age.

[3] Sample size was 1.

Selectivity-related bias from gill-net back-calculation is thus incorporated in the growth parameter estimation. The growth curve calculated from gill-net back-calculation reports unrealistic L_∞ and k. Method 4 (individual back-calculated lengths-at-age derived from all annuli) for the gill net failed because no feasible solution could be found for the parameters of the curve. High variability of back-calculated lengths-at-age derived from all annuli (see standard deviations in Table 3) in the presence of Lee's phenomenon may have been the reason for the unsuccessful attempt to obtain estimates of the parameters of the von Bertalanffy growth curve with this method.

In our study, the growth parameters estimated from individual back-calculated length-at-age derived from the last annulus only (method 3) on purse-seine samples were considered to be the most appropriate for future assessment of the southern Brazilian bluefish stock. This gear is less selective than others and data comprise a wider range of total length groups and age groups.

Changes in Brazilian commercial fishing operations for the bluefish over the last decade have led to changes in the length and age structure of commercial landings. From 1976 to 1983 the maximum age and total length of bluefish in the fishery were 7 years and 630 mm TL, respectively, and less than 5% of fish were 4 years and older (Krug and Haimovici, 1989). Currently the fishery lands fish up to age 10, and 16% of fish are aged 4 and older. The inclusion of older individuals in recent catches is due to the expansion of the fishery into deeper areas. In 1990, the purse-seine fishery began moving to deeper waters, catching a wider range of sizes of bluefish. Hilborn and Walters (1992) have pointed out the necessity to understand fleet dynamics in order to assess the population dynamics of a species.

Our mean back-calculated lengths-at-age (calculated from the last annulus only) are similar to those found by Krug and Haimovici (1989) (except for ages 1 and 3) (Table 5), but estimated growth parameters for the two studies are different. We believe these differences are due to differences in fishing operation between the two periods (fleet operated in shallower water in 1977–83 than in 1992–97) rather than to fishing pressure. Also, the use of individual back-calculated lengths-at-age for the last annulus only (method 3) rather than the mean back-calculated lengths-at-age for all annuli (weighted mean, method 2, Krug and Haimovici, 1989) could have led to such a difference.

Data from the literature indicate considerable variation in length-at–age of bluefish between areas (Table 5). Terceiro and Ross (1993) attributed this variation to a sampling bias in available fish and to the differential proportion of spawned fishes in collected samples (e.g. due to multiple spawning). Champagnat (1983) suggested that differences in size at first maturity could lead to differences in growth parameters. The reported size at first maturity of bluefish varies from 250 mm TL in South Africa (Van der Elst, 1976)

to 430 mm TL in Senegal (Conand, 1975, Champagnat, 1983). In southern Brazil, length at first maturity for bluefish is between 350 to 400 mm TL (Haimovici and Krug, 1992) and this stock is considered to be relatively fast growing.

It is difficult to obtain samples representative of the population's age structure when using a single gear because of the size selectivity of most gears (Bagenal and Tesch, 1978). Also, the ranges of fish length and age represented in a study may affect the estimation of population parameters if they are not representative of the stock (Goodyear, 1995). Our study suggests that the data used for the estimation of the growth parameters for a fish stock should allow for the possible effects of gear selectivity. Data from different gears should be analyzed and should cover as wide an area of the stock distribution as possible. Moreover, the technique applied for estimation of growth parameters should be carefully assessed and the one chosen, strongly justified. Verification of the validity of the back-calculation method and validation of the annual nature of increment formation are also strongly recommended.

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Nursery habitats for ladyfish, *Elops saurus*, along salinity gradients in two Florida estuaries

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Abstract—Ladyfish, *Elops saurus*, are recognized as an estuarine-dependent species, although no published study has described how ladyfish use estuarine habitats. This study found ladyfish to be common throughout Tampa Bay and Indian River Lagoon, Florida. In both estuaries, metamorphosing larvae were collected during several months of the year, but they were most abundant in spring. Length-frequency analyses suggested that age-0 ladyfish grew from 20–30 mm to 200–300 mm standard length during their first year and that at least three age classes were present throughout the year. Age-0 ladyfish followed an ontogenetic migration with regard to salinity. They entered estuaries as metamorphosing larvae and became concentrated in waters of lower than median salinity for both estuaries (23–25 ppt). In Tampa Bay, which had a greater range of salinity than the Indian River Lagoon, age-0 ladyfish were found principally in mesohaline and oligohaline areas; in the Indian River Lagoon, age-0 ladyfish were found in mesohaline and polyhaline waters. In autumn, age-0 ladyfish moved back to higher salinities, into lower parts of the estuaries, and even out to beaches along the Gulf of Mexico. These field observations are consistent with the hypothesis that ladyfish depend on estuaries, specifically positive estuaries, i.e. where freshwater input exceeds evaporative processes. However, published studies also demonstrate that larval ladyfish can metamorphose and juveniles can survive in hypersaline waters; therefore negative estuaries may also serve as suitable nursery habitat. It is not clear how salinity affects ladyfish growth and mortality, and further research should clarify how different types of estuaries (i.e. positive versus negative) contribute to maintaining populations of this fishery species.

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Many fish species use estuaries of the southeastern United States as nurseries (e.g. Skud and Wilson, 1960; Gunter, 1967), but coastal habitat degradation threatens many of the economically important fisheries that rely on estuaries (Gilmore, 1995). The re-enacted Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA) was developed to protect or enhance fisheries habitats, by first requiring information regarding the value of coastal habitats to the survival of marine organisms (e.g. Schmitten, 1996). A simple approach to ranking relative habitat value is to compare intraspecific fish distributions with respect to habitat in different estuaries. Associations between abundance and habitat can assist in predicting the response of coastal fish populations to changes in these habitats. Remarkably, such information is rarely available except for the most economically valuable species (Haedrich, 1983).

Freshwater inflows to estuaries of the southeast United States have been severely altered in the last 150 years and coastal development continues to divert more water away from estuaries (Stickney, 1984). The MSFCMA's Essential Fish Habitat mandate provides a policy framework for identifying the effects of reduced freshwater inflows on estuarine-dependent species. Yet researchers and managers often characterize species as estuarine-dependent more on intuition than rigorous examination of data. Able and Fahay (1998) outlined three criteria for defining estuarine-dependence: 1) predictable use of estuaries, 2) non-use of suitable alternative habitats, and 3) demonstrable effect on a fish population from a loss of estuarine habitat. The first two criteria are best addressed with field studies, but even such simple descriptions of habitat use at a landscape level are lacking for most estuarine fish species (Hoss and Thayer, 1993).

One example of this type of data gap is that for ladyfish, *Elops saurus*, a fishery species that inhabits coastal waters of Florida (Hildebrand, 1963; Murray et al., 1987; FMRI[1]). Ray (1997) listed ladyfish as an estuarine-dependent species but little is known about its use of habitat in coastal waters. Ladyfish are probably considered to be estuarine-dependent because they spawn in offshore waters and metamorphosing larvae and juveniles are found inshore (Hildebrand, 1943; Gehringer, 1959; Eldred and Lyons, 1966). However, ladyfish tolerate a wide range of salinities (Alikunhi and Rao, 1951; Gunter, 1956; Gehringer, 1959; Bayly, 1972). Numerous studies have reported the occurrence of small juveniles in mesohaline or lower-salinity waters (<18 ppt), which is consistent with an estuarine-dependent life history (Tagatz and Dudley, 1961; Gunter and Hall, 1965; Zilberberg, 1966; Tagatz, 1968; Tagatz and Wilkens, 1973; Sekavec, 1974; Govoni and Merriner, 1978 [and references within]; Thompson and Deegan, 1982; Peterson and Ross, 1991). Moreover, large ladyfish are present throughout

[1] FMRI (Florida Marine Research Institute): www.floridamarine.org

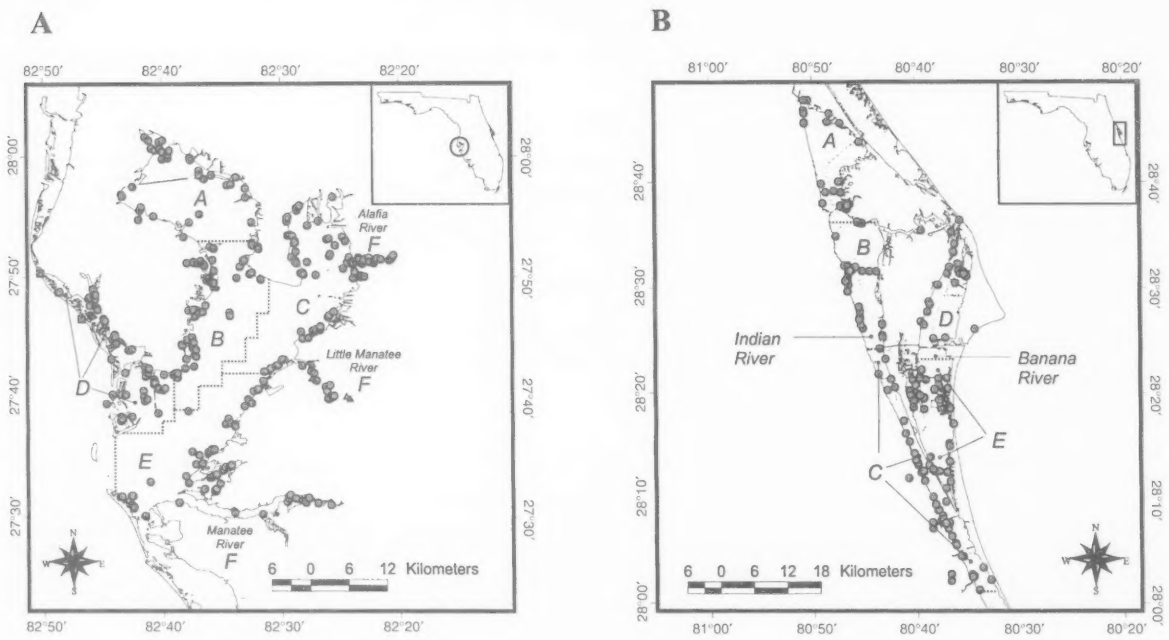

Figure 1

Sampling areas and locations where at least one ladyfish, *Elops saurus*, was collected for (**A**) Tampa Bay and (**B**) the Indian River Lagoon. Different symbols are used to represent collections in Tampa Bay: the bay-wide program (circles), the Little Manatee River program (triangles), and the Gulf Beach program (squares). Lines and letters (A–F) designate the sampling zones in each system. See text for further definition of zones and Tables 1–2 for details of all collections.

the year in polyhaline and marine (>18 ppt) waters such as Florida Bay, Biscayne Bay, and the Indian River Lagoon (Low, 1973; Sogard et al., 1989a, 1989b; Tremain and Adams, 1995). Other studies, however, have reported presumptive age-0 juveniles (Harrington and Harrington, 1961; Kristensen, 1964), older subadults (Carles, 1967), or both (Simmons, 1957; Roessler, 1970; Brockmann, 1974) in hypersaline waters (>35 ppt). Thus, age-0 ladyfish may be seeking some condition other than specific salinities.

The purpose of our study was to determine whether age-0 ladyfish make predictable size-specific seasonal movements that would identify them as an estuarine-dependent species. Because salinity is part of the definition of an estuary (Pritchard, 1967), a landscape approach was used to follow ladyfish movements with respect to salinity within Tampa Bay and the Indian River Lagoon. If age-0 ladyfish select low-salinity waters, then we predicted that they would be found in lower than average salinity waters for each estuary. Furthermore, if age-0 ladyfish select low-salinity habitats, then we predicted that they would be more abundant in the lower salinity areas of Tampa Bay because this bay has a wider salinity range to select than that for the Indian River Lagoon. We also examined length-frequency data to make preliminary assessments of age-class composition and growth rates of ladyfish within estuaries. Although several publications have discussed the ecology of ladyfish in estuaries (e.g. Springer and Woodburn, 1960 and citations above), to our knowledge the data treatment in our study is the most comprehensive for this species.

Methods

Study sites

Data from Tampa Bay, on central Florida's west coast, were compared with data from the Indian River Lagoon, on central Florida's east coast (Fig. 1). Both water systems are located at similar latitudes, so they are subject to similar temperature cycles. Both systems are positive estuaries (i.e. freshwater input exceeds evaporative processes; Pritchard, 1967), but they have distinctive salinity regimes (Fig. 2). Tampa Bay is a drowned river system with considerable freshwater input and separate satellite barrier-island embayments, whereas the Indian River Lagoon is largely a series of barrier-island embayments with few inlets and no major rivers (Comp and Seaman, 1985).

Bay-wide survey—Tampa Bay We examined survey data for Tampa Bay fishes collected from 1989 to 1995 by staff of the Florida Marine Research Institute (FMRI). The sampling design incorporated both fixed sampling stations and locations assigned in a stratified random manner (McMichael et al., 1995). Tampa Bay was stratified into six major zones (Fig. 1A): the upper bay (A), the western bay (B),

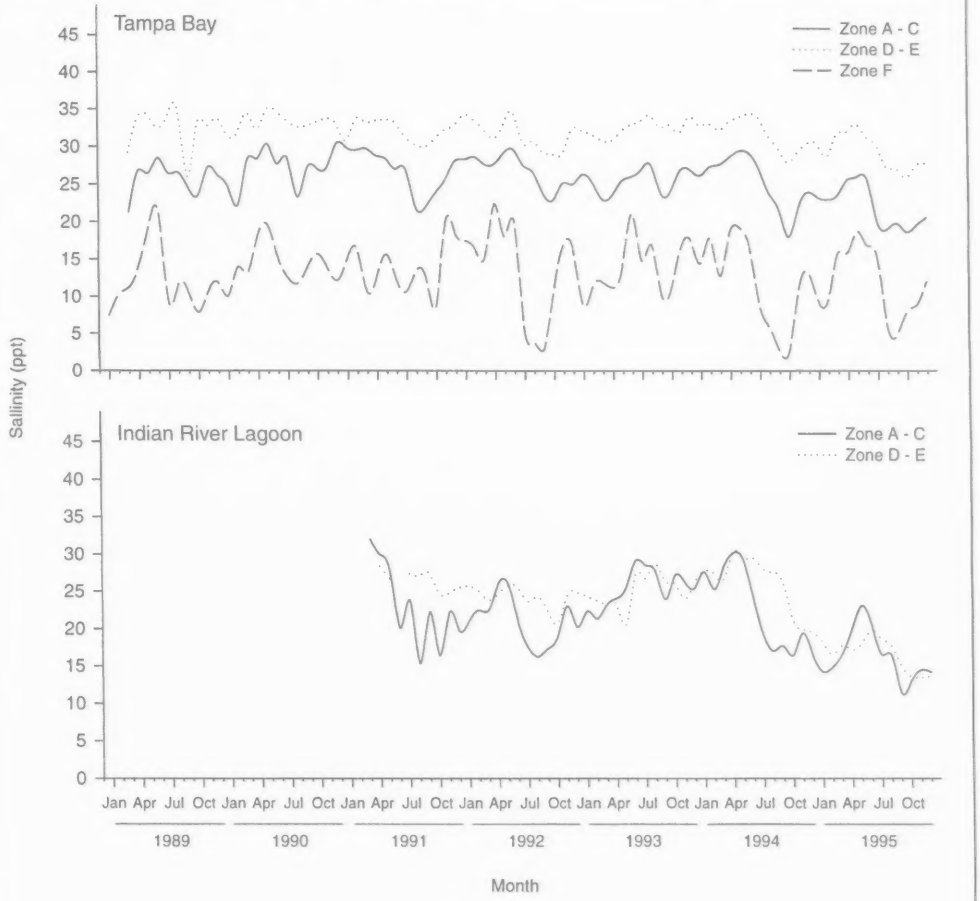

Figure 2

Salinity trends for Tampa Bay (1989–95) and the Indian River Lagoon (1991–95). Mean monthly salinity values were calculated based on all available data and fitted with spline curves. Some sampling zones were combined for graphical simplicity (compare with Fig. 1). Indian River Lagoon 1990 data, which were collected only during the spring (Mar–May) and fall (Sep–Nov) months, were not included.

the eastern bay (C), the satellite embayments (D), the lower bay (E), and the principal rivers (F). Each zone was stratified further by a 1-square-nautical-mile grid system. Sampling at fixed stations occurred monthly, but stratified random sampling was conducted principally in spring and autumn when many species recruit to Florida estuaries.

Sampling gear included seines, trawls, block nets, and gill nets (Table 1). A 21.3-m center-bag seine was deployed in one of three different ways: 1) it was hauled along the shoreline across an area of about 340 m^2 ("beach sets"); 2) it was deployed from a boat in a semicircular pattern in river zones and the mean area swept was 70 m^2 ("boat sets"); 3) or it was set away from the shoreline and hauled into the current across an area of about 140 m^2 ("offshore sets"). A much larger seine (183-m) was also deployed from a boat in a semicircular pattern. A 61-m block net was set against seawalls or mangroves of inundated shorelines at high tide, and fish were collected at the ensuing low tide. Otter trawls were towed for 10 min in bay zones (zones A–E) and for 5 min in river zones (zone F) at an approximate speed of 0.6 m/s. A 184-m gill net with four 46-m panels (75-, 100-, 125-, and 150-mm mesh) and a similar 198-m gill net that included a 15-m section of 50-mm mesh were used. These gill nets were set perpendicular to shore, four nets at a time, so that two nets with the larger mesh were oriented inshore and two nets were oriented in the opposite direction.

Little Manatee River–Tampa Bay In addition to sampling the Little Manatee River as part of the bay-wide survey, FMRI staff completed an independent survey of this river

Table 1

Sources and details of data examined from Tampa Bay: sampling gear used (with mesh size); geographic zones sampled (see Fig. 1A); diel periods (D=day, N=night, C=crepuscular); months (1=January); years sampled during 1989–95; number of ladyfish collected (C (c)[4]); and total number of sets with each net (f). The bay-wide survey of Tampa Bay included a stratified, random survey design and a fixed-station sampling design. The special survey of the Little Manatee River and the Gulf Beach survey used fixed stations.

Gear	Mesh (mm)	Zones	Period	Months	Years	C (c)[4]	f
Bay-wide fixed-station survey							
21.3-m seine (340 m^2)[1]	3.2	A, (B–D),[2] F	D	1–12	1989–95	60	377
21.3-m seine (70 m^2)[1]	3.2	F	D	1–12	1989–95	942 (6)	1818
21.3-m seine (140 m^2)[1]	3.2	A–E	D, N, C[2]	1–12	1989–95	22	2081
183-m seine	38.5	B–F	D	1–12	1992,[2] 1993–95	1585	458
61-m block net	3.2	D	D, N, C[2]	1–12	1990,[2] 1991–92	148	210
6.1-m trawl	3.2[3]	B–F	D	1–12	1989,[2] 1990–95	32 (2)	2249
Bay-wide, stratified random station survey							
21.3-m seine (340 m^2)[1]	3.2	A–E,F[2]	D, N, C	3–6, 9–12	1989–95	35	1146
21.3-m seine (70 m^2)[1]	3.2	(A–E),[2] F	D, N, C	3–6, 9–11, 12[2]	1989–95	31	639
21.3-m seine (140 m^2)[1]	3.2	A–F	D, N, C	3–5, 6,[2] 9–12	1989–95	16	1464
6.1-m otter trawl	3.2[3]	A–F	D, N, C	3–6, 9–12	1989–95	3 (5)	2538
184-m gill net	75–150	A–E, F[2]	N, C	3–6, 9–12	1989–93	952	427
198-m gill net	50–150	A–E	N, C	3–5, 9–11	1994–95	583	160
Little Manatee River survey							
9.1-m seine	3.2	—	D	1–12	1989–91	15	77
22.9-m seine	3.2	—	D	1–12	1988–91	115	1460
120-m seine	3.2	—	D	1–12	1990–91	148 (1)	89
Gulf Beach survey							
22.9-m seine	3.2[3]	—	D	1–12	1992–94	156	435
Total						4843 (14)	

[1] Value in parentheses indicates area swept by each haul; see text for further details.
[2] Less than 30 tows for this sampling unit.
[3] Minimum mesh size used.
[4] C = late-metamorphic, juvenile, and older stages; (c) = early- or mid-metamorphic (leptocephalus) larvae.

between January 1988 and December 1991 (Table 1; Fig. 1A). Samples were collected biweekly with a 22.9-m seine at six fixed shoreline stations located between the river mouth and the freshwater zone, with 2–3 seine hauls per station. Supplemental samples were collected with 9.1- and 22.9-m seines at two additional stations from January 1989 to June 1991 and with a 120-m seine at five fixed sites near the river mouth from March 1990 to November 1991.

Gulf beaches—Tampa Bay In a third independent survey by FMRI staff, two beach sites were sampled along the Gulf of Mexico coast of Pinellas County, FL (Table 1; Fig. 1A). Samples were collected biweekly with a 22.9-m seine from September 1992 to November 1994 at Treasure Island and from August 1993 to November 1994 at Indian Shores. Five hauls were made in the surf zone at each site during a single day; each haul began 50 m from the water's edge and proceeded perpendicular to shore.

Lagoon-wide Survey—Indian River Lagoon We also examined data from an FMRI survey of the Indian River Lagoon fishes. The same general sampling design and gear were used in this survey and the bay-wide survey of Tampa Bay (see above; Tables 1–2; Tremain and Adams, 1995). Although the Indian River Lagoon survey started slightly later than the Tampa Bay survey, they were largely contemporaneous (1990–1995). The northern Indian River Lagoon system is a complex of the Indian River basin (zones A–C) and the Banana River basin (zones D–E; Fig. 1B). The sampling program in the Indian River Lagoon differed slightly from that in Tampa Bay. In the Indian River Lagoon, neither the 183-m seine nor block nets were used; gill nets were used at fixed stations and stratified-random locations, and fewer total hauls were made with most types of sampling gear because portions of this program started one or two years later than Tampa Bay's program.

Abundance and size of ladyfish

Monthly relative abundance was calculated as the mean number of ladyfish per haul (including hauls with no ladyfish) for each gear type used in each survey. Data from the stratified random programs were not included in calculations of monthly relative abundance because stratified

Table 2

Sources and details of data examined from the Indian River Lagoon: sampling gear used (with mesh size); geographic zones sampled (see Fig. 1B); diel periods (D=day; N=night; C=crepuscular); months (1=January); years sampled during 1989–95; number of ladyfish collected (C (c)[4]); and total number of sets with each net (f).

Gear	Mesh (mm)	Zones	Period	Months	Years	C (c)[4]	f
Lagoon-wide fixed-station survey							
21.3-m seine (340 m^2)[1]	3.2	A, C–D	D	1–12	1991–95	273 (367)	619
21.3-m seine (70 m^2)[1]	3.2	B,[2] C, E[2]	D	(1–12)[2]	1991–95	403 (67)	235
21.3-m seine (140 m^2)[1]	3.2	A, C–E	D, C[2]	1–12	1991–95	42 (96)	668
6.1-m trawl	3.2[3]	C–E	D	1–3 (4–5),[2] 6–12	1991,[2] 1992–95	7 (1)	617
184-m gill net	75–150	A, D	C	(1–12)[2]	1991–93, 1994[2]	95	202
198-m gill net	50–150	A–D	C, N[2]	(1–12)[2]	1994–95	153	126
Lagoon-wide stratified, random station survey							
21.3-m seine (340 m^2)[1]	3.2	A–E	D, N, C	3–5, 9–11	1990–95	232 (218)	977
21.3-m seine (70 m^2)[1]	3.2	D[2]	D,[2] C[2]	5[2]	1990[2]	0	6
21.3-m seine (140 m^2)[1]	3.2	A–E	D, N, C	3–5, 9–11	1990–95	46 (61)	1286
6.1-m otter trawl	3.2[3]	A–E	D, N, C	3–5, 9–11	1990–95	1 (5)	1501
184-m gill net	75–150	A–E	N, C	3–5, 9–11	1990–93	392	319
198-m gill net	50–150	A–E	N, C	3,[2] 4, 5,[2] 9,[2] 10,[2] 11	1994–95	457	168
Total						2101 (815)	

[1] Value in parentheses indicates area swept by each haul; see text for further details.
[2] Less than 30 tows for this sampling unit.
[3] Minimum mesh size used.
[4] C = late-metamorphic, juvenile, and older stages; (c) = early- or mid-metamorphic (leptocephalus) larvae.

random sampling did not occur in all months of the year. Geographic distributions of age-0 ladyfish were also plotted for each of four seasons. For such maps, relative abundance was calculated as the number of age-0 ladyfish per haul. Values for all positive catches were categorized into four quartile classes (≤25th, 26–50th, 51–75th, and >75th percentile) before plotting, to standardize the data among gear types and estuary. Maximum size criteria were adjusted for each season to exclude fish older than age 0.

Fish size was measured and reported as standard length (SL) in mm. At least 20 randomly selected ladyfish per sample were measured and unmeasured fish were proportionally adjusted for the length-frequency plots to reflect the size structure of the entire sample(s). Sizes from the literature are also reported as SL and were converted when necessary by using the equations $SL = -0.772 + 0.787$ (*total length*), or $SL = -2.46 + 0.943$ (*fork length*); each equation was based on least squares regressions of measurements from 75 ladyfish that ranged from 39 to 475 mm SL (r^2=0.99). Mean salinity at capture was calculated for each 25-mm-SL interval, and for each estuary separately, by using the weighted formula

$$\bar{Y}_w = \left(\sum^{n} w_i Y_i\right) \Big/ \sum^{n} w_i ,$$

where w_i = the number of ladyfish per 25-mm-SL interval for collection i;
Y_i = the salinity measured for collection i; and
n = the total number of collections with fish in that 25-mm-SL interval for that estuary.

We identified early life stages of ladyfish from their size and appearance. The following criteria and general terminology are from Gehringer (1959). Early-metamorphic larvae have a leptocephalus form, with a clear and laterally compressed body. Early-metamorphic larvae shrink as they develop from about 45 to 25 mm SL. Mid-metamorphic larvae are generally less than 25 mm SL. Mid-metamorphic larvae shrink to about 18 mm SL and then grow to about 25 mm SL; they lose the leptocephalus form by the end of this stage. Late-metamorphic larvae have a juvenile form and grow from about 25 mm to 60 mm SL. After 60 mm the age-0 fish are referred to as juveniles. Older age classes (i.e. age-1 or age-2+) are defined by size, as inferred from length-frequency analysis. The complete size and age range of ladyfish in estuaries is not defined in Gehringer or other published literature but appears to be largely restricted to immature fish.

Results

Tampa Bay

In the bay-wide survey, 4422 ladyfish were captured in 7525 21.3-m-seine hauls, 458 183-m-seine hauls, 210

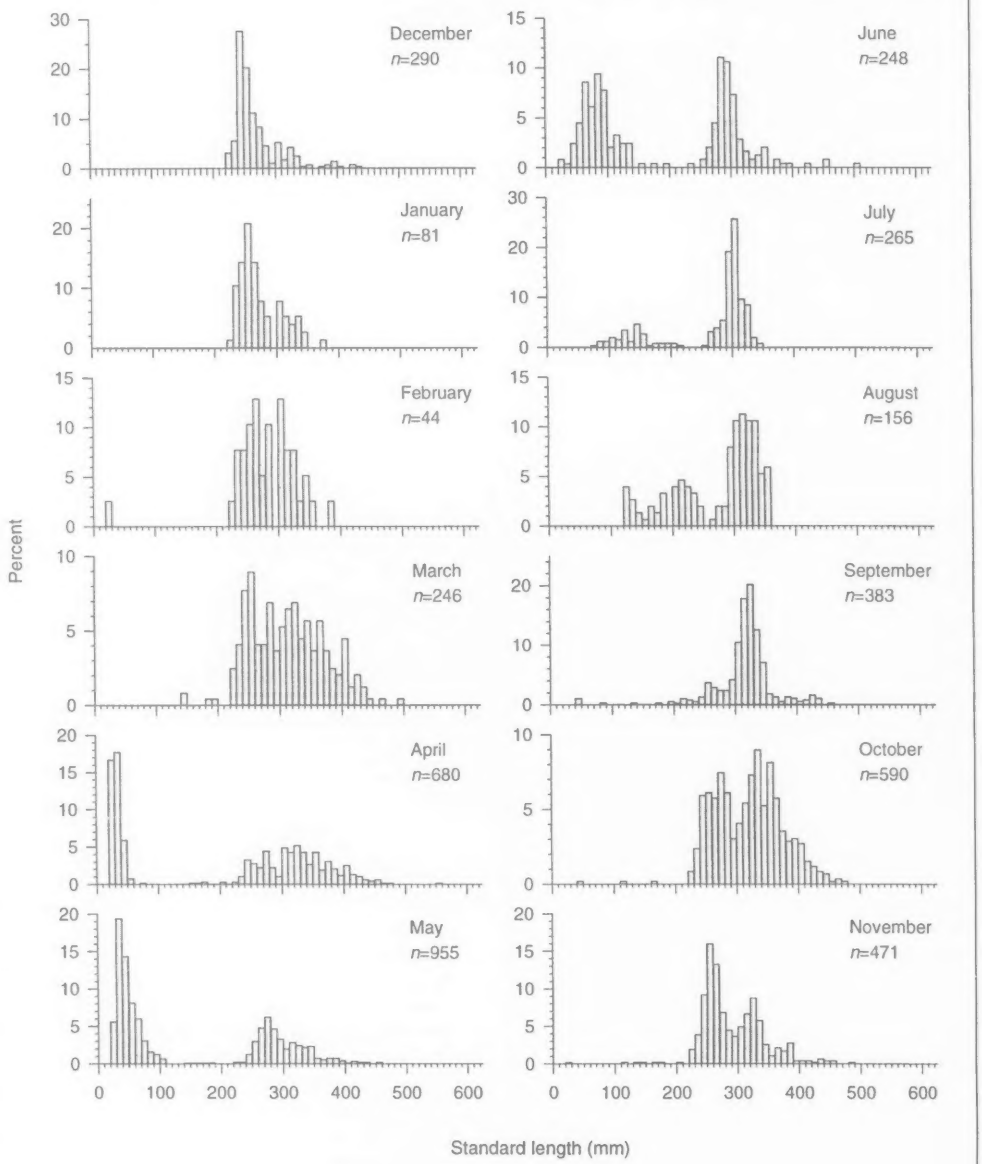

Figure 3

Monthly length-frequency of ladyfish, *Elops saurus*, in Tampa Bay. Early- and mid-metamorphic larvae were excluded because length decreases as they develop. Bay-wide data for all years (1989–95) from all zones (A–F) are plotted. n = number of fish sampled.

blocknet sets, 4787 trawl hauls, and 587 gillnet sets (Table 1). Generally, a new cohort of late-metamorphic larvae first appeared in April (Figs. 3 and 4A). Only 13 early- or mid-metamorphic larvae were collected (26–34 mm SL) in the bay-wide survey (n=14 for all Tampa Bay samples). Although the arrival of metamorphosing larvae (largely late-metamorphic stages) was concentrated in April, isolated individuals 26–41 mm SL were collected as early as February (1989) or March (1994, 1995) or as late as September (1994), October (1993), or

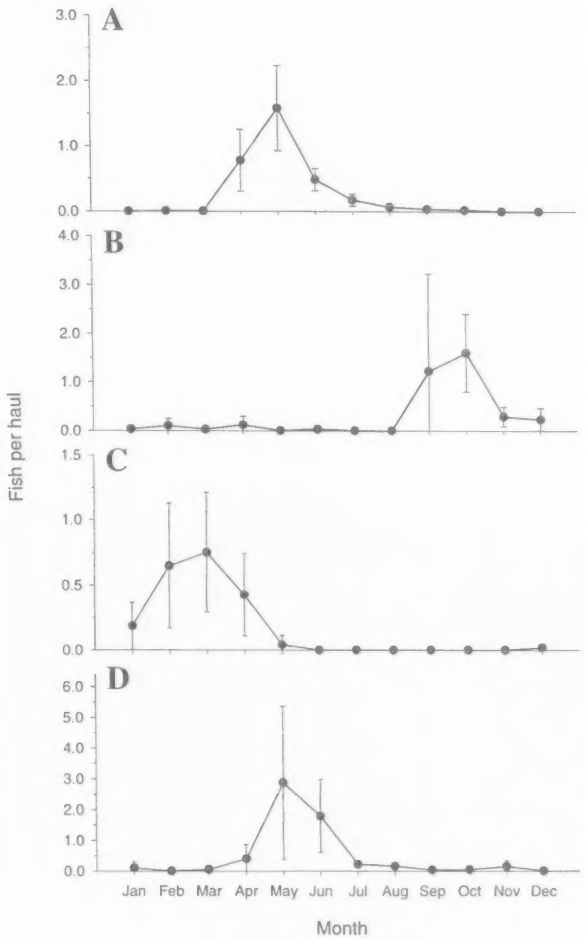

Figure 4

Monthly catch-per-unit-of-effort (mean number per haul ±2 standard error bars) for ladyfish, *Elops saurus*, collected with 21-m and 23-m seines at fixed stations during 1989–1995 in Tampa Bay or 1990–95 in the Indian River Lagoon. (**A**) Little Manatee River (early- and mid-metamorphic larvae are excluded); (**B**) Gulf of Mexico beaches; (**C**) Indian River Lagoon leptocephali (i.e. only early- and mid-metamorphic larvae); (**D**) Indian River Lagoon late-metamorphic and juvenile ladyfish (i.e. early- and mid-metamorphic larvae excluded).

November (1990). All ladyfish ranged in length from 20 to 550 mm SL (Fig. 5). Length-frequency analyses suggested that there were three age classes: age-0 fish overwintering at 200–300 mm SL, age-1 fish overwintering at 300–400 mm SL, and age-2+ at sizes of 400 mm SL or larger (Fig. 3).

Nearly all ladyfish <200 mm SL were collected in mesohaline and oligohaline areas of rivers (zone F; Fig. 6). Early- and mid-metamorphic larvae were collected at polyhaline salinities (mean=20 ppt, Fig. 7); these larvae had probably entered Tampa Bay recently. In Little Manatee River, ladyfish were most common (59.7% of the total individuals) in the mesohaline zone (5.1–18 ppt), less common in the oligohaline zone (31.9%; 0.5–5.0 ppt), uncommon in the polyhaline zone (6.7%; 18.1–30 ppt), and rare in fresh water (1.7%; <0.5 ppt). Abundance in oligohaline waters peaked during June, about one month later than peak abundance in mesohaline waters.

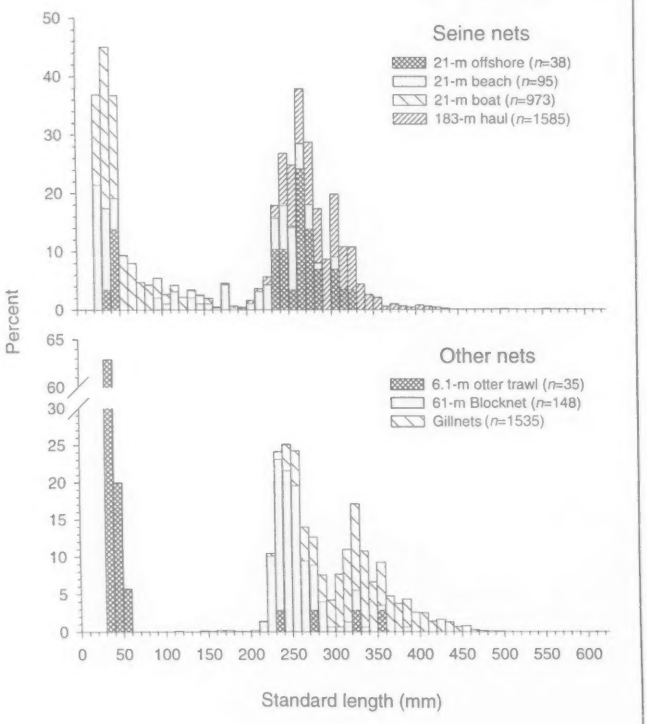

Figure 5

Length-frequency of ladyfish, *Elops saurus*, in Tampa Bay, plotted by sampling gear type. Early- and mid-metamorphic larvae were excluded. Data for all years (1989–95) and all zones (A–F) of bay-wide sampling were plotted. *n* = number of fish sampled.

During autumn, when age-0 fish were <300 mm SL, they began to move out of the rivers (Fig. 8). Fish measuring 170–360 mm SL (5–95 percentile; n=55 fish) were found at the mouth of the Little Manatee River during the winter. Large concentrations of age-0 ladyfish were also found at other river mouths, near the dredged (>12 m) portions of the Alafia River, and near the mouth of Tampa Bay. There was a sudden pulse of ladyfish along Gulf of Mexico beaches during September–October (Fig. 4B). These were juvenile age-0 ladyfish measuring 219–260 mm SL (5–95 percentile; n=126). Only 2 of 158 individuals collected at Gulf beaches were smaller than 178 mm SL. The absence of larval ladyfish in shallow Gulf beach habitats contrasted strongly with the abundance of larval ladyfish in riverine habitats of Tampa Bay.

Indian River Lagoon

A total of 2916 ladyfish were captured in 3791 21.3-m seine hauls, 2118 trawl hauls, and 815 gillnet sets (Table 2). The seasonal pattern of arrival was similar to that in Tampa Bay. Early- and mid-metamorphic larvae were much better represented (28% or n=815) in the Indian River Lagoon than in Tampa Bay (0.2% or n=14), and these leptocephali were present from at least December to May (Fig. 4C). Late-metamorphic larvae were found in many months throughout the year, but they were most abundant during spring (Figs. 4D and 9). All ladyfish collected in the Indian River Lagoon ranged from 20 to 600 mm SL. Age-0 fish reached a modal length during winter (i.e. 250–270 mm SL), similar to that observed in Tampa Bay. Age-1 ladyfish appeared to overwinter at a mode of about 350 mm SL, and a small number of age-2+ were probably present as postulated for Tampa Bay.

Ladyfish <100 mm SL were more common in the Indian River basin than in the Banana River basin, and fish >300 mm SL were more common in the Banana River basin than in the Indian River basin (Fig. 10). Juveniles first occupied upper mesohaline and lower polyhaline salinities at sizes of about 75 mm SL, and they remained at such salinities until reaching about 300 mm SL (Fig. 7). In the Indian River Lagoon, the mean salinity occupied by ladyfish 75–125 mm SL was about 5 ppt higher than in Tampa Bay, but mesohaline and oligohaline habitats are less common in

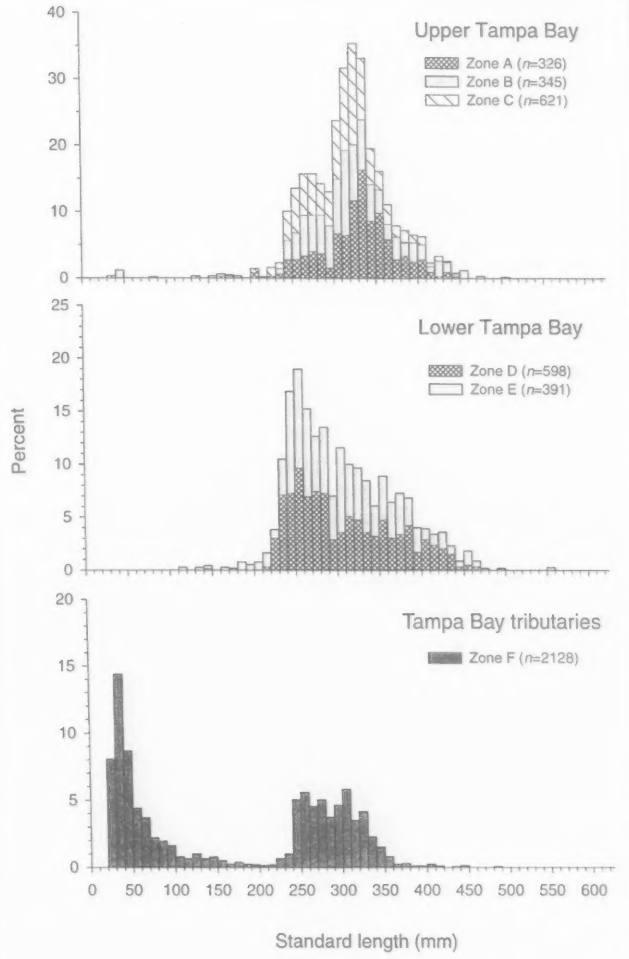

Figure 6

Length frequency of ladyfish, *Elops saurus*, plotted by sampling zones in Tampa Bay. Early- and mid-metamorphic larvae were excluded. Data for all years (1989–95) of bay-wide sampling were plotted. n = number of fish sampled.

Indian River Lagoon than in Tampa Bay. Overall, however, ladyfish were broadly distributed in both the Indian River and Banana River basins throughout the year (Fig. 11).

Discussion

Ladyfish were common, widely distributed, and fast growing in both Tampa Bay and the Indian River Lagoon. Metamorphosing larvae and overwintering juveniles were linked together in a single study by using multiple sampling gears. Ladyfish ages inferred from length frequencies indicated that few fish older than 2–3 years were present in either estuary. Over a thousand ladyfish gonads from Tampa Bay and the Indian River Lagoon were examined macroscopically, and nearly all fish were found to be immature (McBride, pers. obs.). Overall, our observations agree with previous reports that ladyfish arrive in coastal embayments as metamorphosing larvae and leave after about 2–3 years to mature and eventually spawn at sea. Carles' (1967) samples from a hypersaline Cuban lagoon contained only immature age 1–3 fish (115–375 mm SL). Others who have suggested that ladyfish mature at sea, where they reach a maximum age of about 6 years and a maximum length of 570–660 mm SL, include Hildebrand (1963), Palko (1984), and Santos-Martinez and Arboleda (1993).

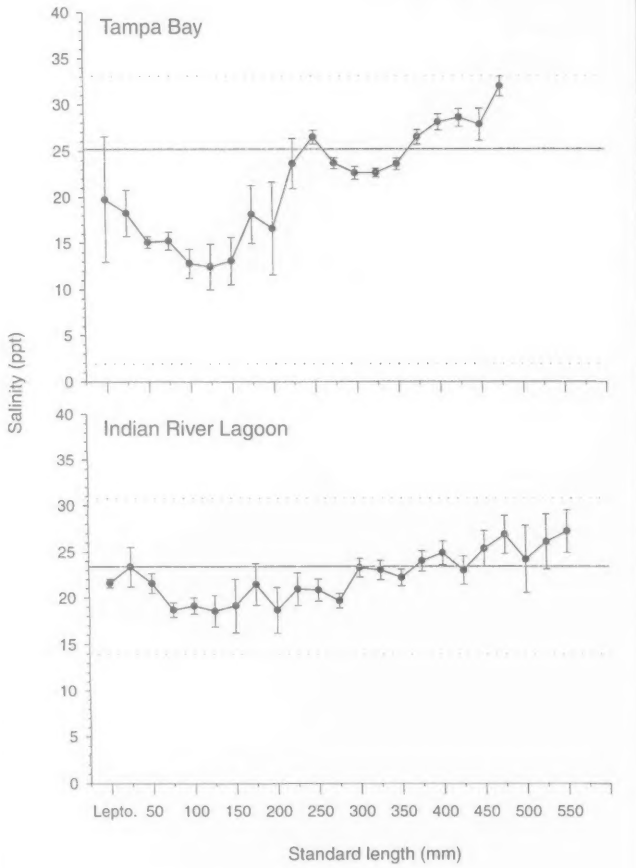

Figure 7

Density-weighted mean salinity at capture for different sizes and stages of ladyfish, *Elops saurus*, in Tampa Bay (1989–95) and Indian River Lagoon (1990–95). Early- and mid-metamorphic larvae are indicated as "Lepto." Error bars represent 2 standard errors. The solid horizontal line represents the median salinity value, and the dotted horizontal lines represent the 10^{th} and 90^{th} salinity percentiles, based on all samples (with or without ladyfish) from each system.

Ladyfish in both Florida estuaries followed a similar sequence of size-specific movements with respect to salinity. Soon after metamorphosis and for much of their first year, ladyfish occupied waters that were lower than the median salinity of each estuary. Following their first winter, ladyfish occupied higher than median salinities. The available salinity range was wider in Tampa Bay and ladyfish were found in a wider range of salinity in Tampa Bay than ladyfish in the Indian River Lagoon. Some details of these movements deserve further investigation. For example, as juveniles grew to about 150 mm SL during summer they were found in progressively lower salinities. However, they may have passively remained in shallow-water habitats where salinity was decreasing because of summer rains, instead of actively selecting lower salinity waters. We also did not examine other factors that may covary with salinity; therefore we cannot rank the effect of salinity in relation to other variables. In one such simultaneous analysis, Friedland et al. (1996) showed that juvenile menhaden (*Brevoortia tyrannus*) select waters of high chlorophyll-*a* levels more consistently than a specific salinity value.

Temperature presumably affects ladyfish distributions as well. During colder than average winters, ladyfish have experienced hypothermal mortality in both Tampa Bay (Springer and Woodburn, 1960) and the Indian River Lagoon (Snelson and Bradley, 1978). We observed ladyfish moving into deeper water, to river mouths, to the lower

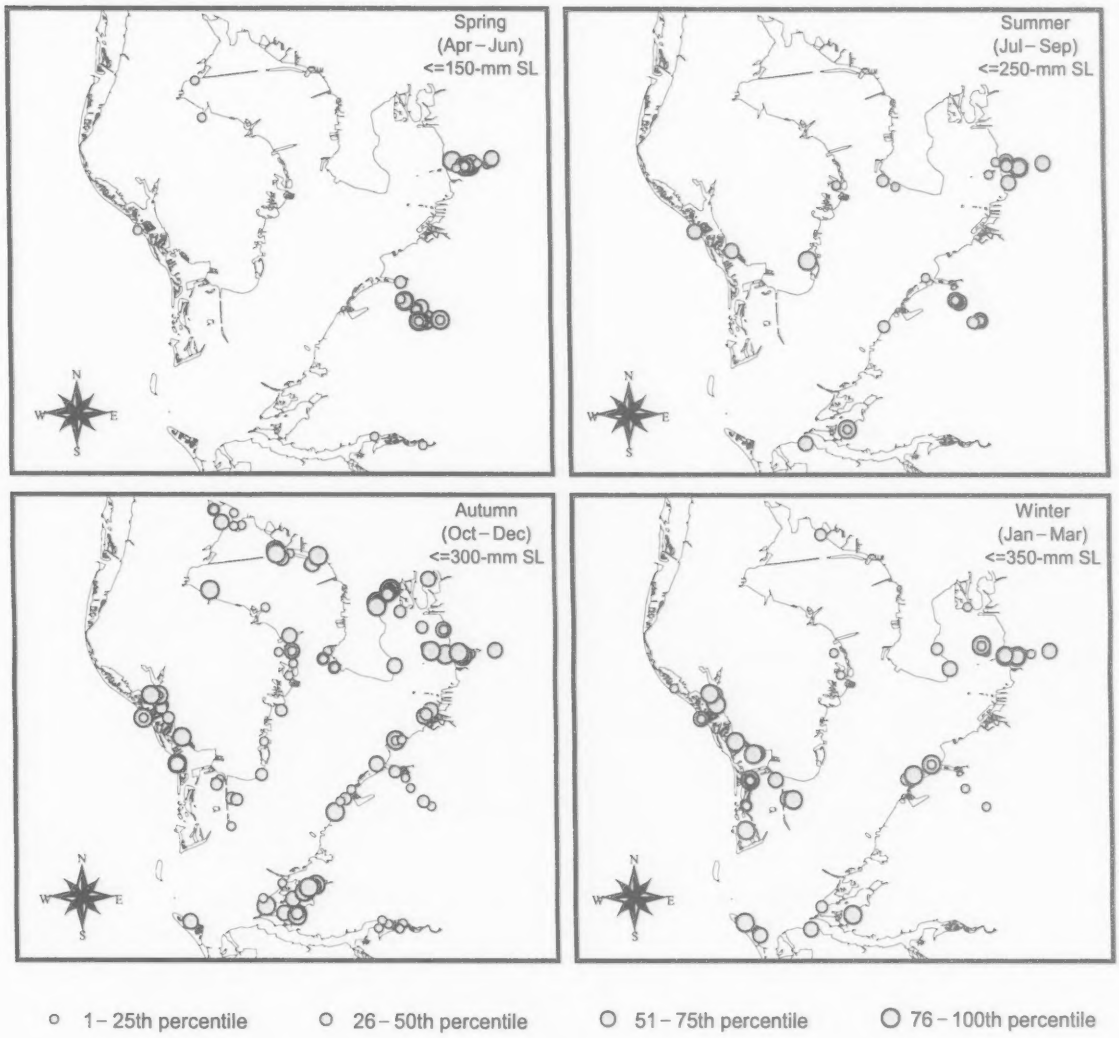

Figure 8

Seasonal distribution plots for age-0 ladyfish, *Elops saurus*, collected in Tampa Bay, 1989–95. Catch-per-unit-of-effort data from all positive catches of age-0 ladyfish were plotted as four quartile classes to standardize the data among gear types; increasing symbol size indicates higher catch per unit of effort. Smaller-size, age-1 ladyfish were excluded by increasing the size range for age-0, as indicated in each plot, from spring to winter.

part of Tampa Bay, and out into Gulf of Mexico waters during colder months. Ladyfish may be selecting these areas for overwintering because such areas are less affected by atmospheric cold fronts. The critical temperatures for ladyfish survival have not been examined in the laboratory, but the above field studies suggest that they could be as low as 10°C. Further work remains on the processes of habitat selection by ladyfish within estuaries.

This description of ladyfish early life history satisfies the first two of three predictions for an estuarine-dependent species: 1) predictable use of estuarine habitats, 2) absence of fish in suitable alternative habitats, and 3) demonstration of a negative population impact caused by the loss of estuarine habitats. Age-0 ladyfish followed an ontogentic migration along a salinity gradient and larval stages of ladyfish were absent from Gulf beaches. The third prediction, that of a negative impact on the population by the absence of habitat, was not supported by findings in our study. We anticipated that salinity differences between Tampa Bay and the Indian River Lagoon might affect growth rates, either due to osmoregulatory stress or perhaps to other correlated factors. Metamorphosing larvae moved into both estuaries at about the same time (i.e. in spring) but age-0 fish attained similar lengths

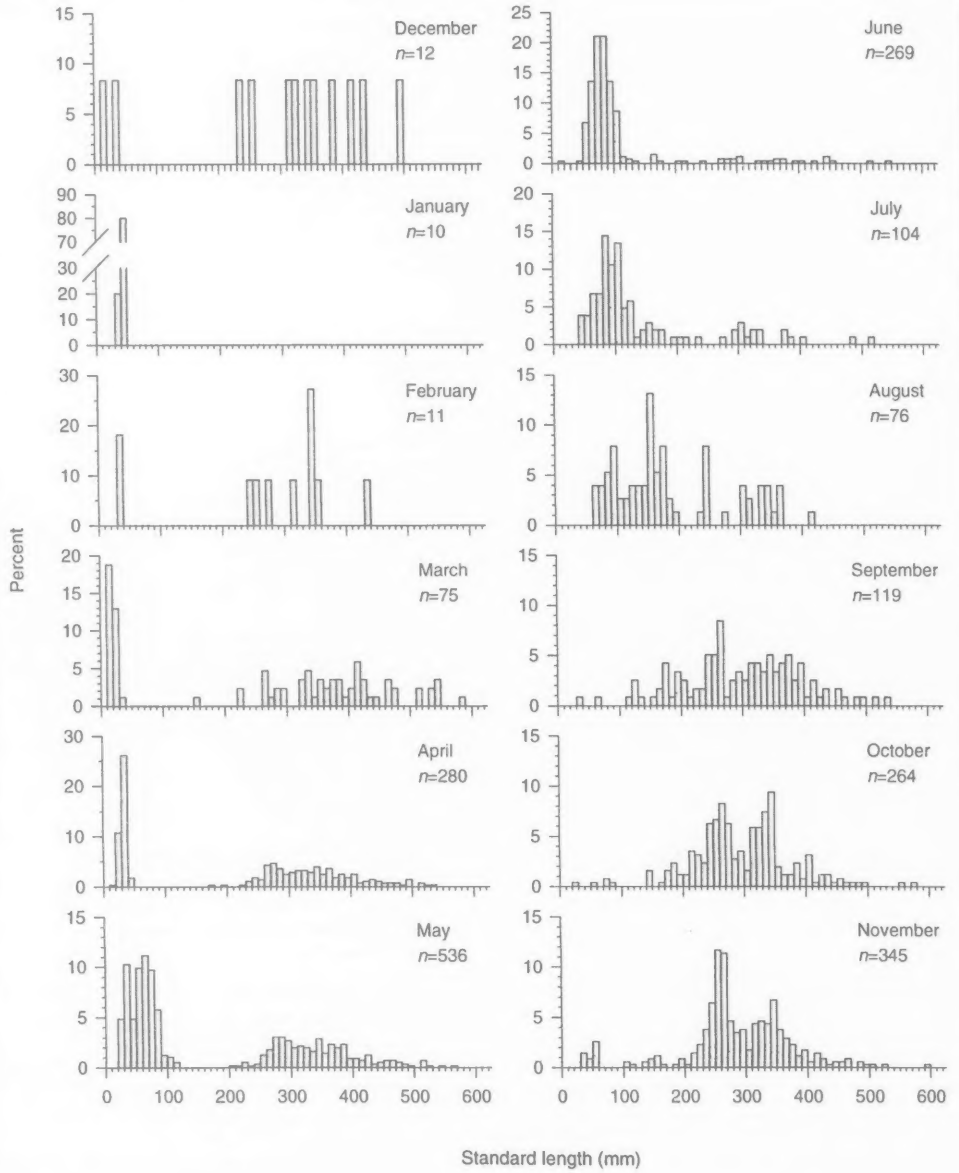

Figure 9

Monthly length frequency of ladyfish, *Elops saurus*, in the Indian River Lagoon. Early- and mid-metamorphic larvae were excluded because length decreases as they develop. Data from all years (1990–95), all gear types, and sampling zones (A–E) were plotted. *n* = number of fish sampled.

(250–300 mm SL) by winter. In Florida Bay, age-0 ladyfish entered estuaries at similar stages during spring and reached similar overwintering sizes as well (Roessler, 1970). In constrast, Carles (1967) estimated much slower growth rates for ladyfish residing in hypersaline lagoons of Cuba, with sizes at annulus formation of 130 mm SL at annulus I, 195 mm SL at annulus II, and 247 mm SL at annulus III. If the growth increments observed by Carles (1967) are indeed annual (he used unvalidated scale annuli), then hypersaline (>35 ppt) conditions may

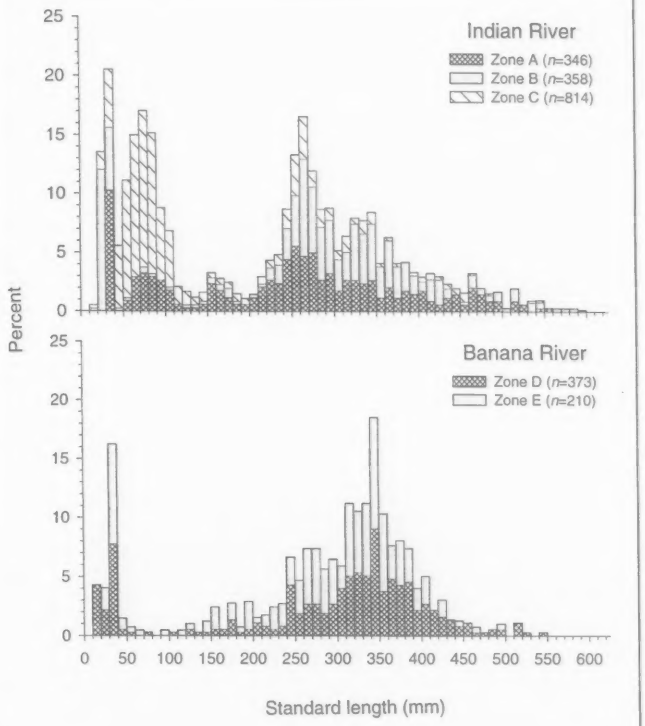

Figure 10

Length frequency of ladyfish, *Elops saurus*, plotted by sampling zones in the Indian River Lagoon. Banana River and Indian River basins are identified in Figure 1B. Early- and mid-metamorphic larvae were excluded. Data from all years (1990–95) and gear types were plotted. n = number of fish sampled.

reduce ladyfish growth rates. The timing of ingress into the Cuban estuaries by metamorphosing larvae was not reported by Carles (1967); therefore it is not clear if overwintering sizes are comparable (i.e. if they represent the same seasonal growth period). If ingress into the Cuban estuaries occurred later than spring (i.e. when ladyfish entered Florida estuaries), then this would shorten the length of the growing season and could explain Carles' (1967) results. This preliminary attempt to link salinity to growth rate, although suggestive, requires verification of reduced growth rates in hypersaline conditions. Experimental studies to determine the optimal salinity for ladyfish growth and survival will clarify whether ladyfish benefit by actively selecting low-salinity habitats. Such information would be the last step in demonstrating that ladyfish depend on estuaries and for showing the relative value of positive estuaries (with low salinity areas) to negative estuaries (with hypersaline conditions) for ladyfish populations. It would also be the next step for defining the essential fish habitat of ladyfish and for predicting the effects of changes in estuarine salinity on this fishery species.

Acknowledgments

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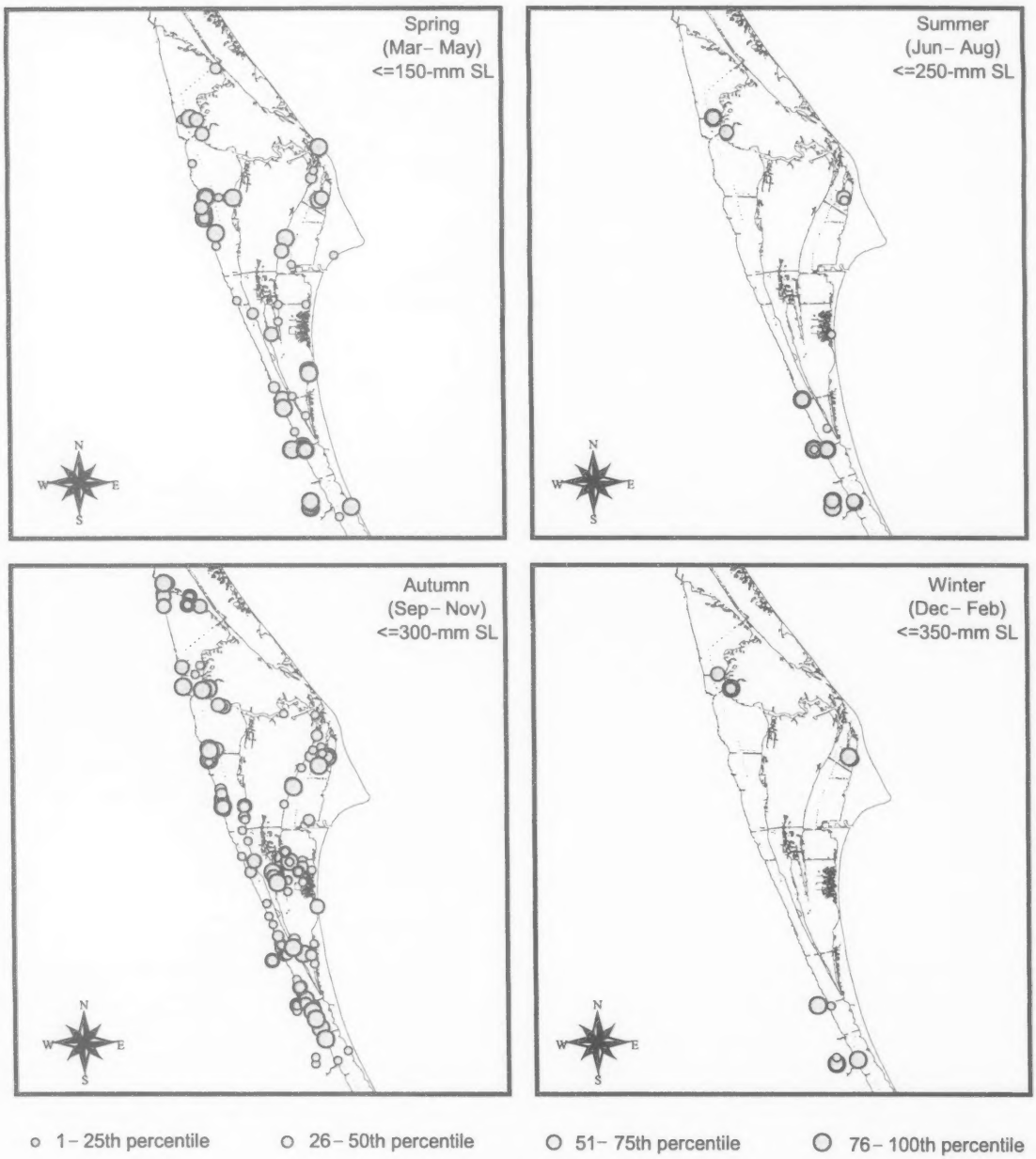

Figure 11

Seasonal distribution plots for age-0 ladyfish, *Elops saurus*, collected in the Indian River Lagoon, 1991–95. Banana River and Indian River basins are identified in Figure 1B. Catch-per-unit-of-effort data from all positive catches of age-0 ladyfish were plotted as four quartile classes to standardize the data among gear types; increasing symbol size indicates higher catch per unit of effort. Smaller-size, age-1 ladyfish were excluded by increasing the size range for age-0, as indicated in each plot, from spring to winter.

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The validity of the fin-ray method of age determination for lingcod (*Ophiodon elongatus*)

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Abstract—Oxytetracycline (OTC) injections were used in a mark-recapture experiment undertaken to validate the fin-ray method of age determination of lingcod (*Ophiodon elongatus*). In most cases, the number of annuli that formed beyond the OTC mark corresponded to the number of years at liberty. Expected and interpreted annuli counts from fin rays matched in 94%, 92%, 91%, and 75% of lingcod at liberty for one, two, three, and four years, respectively. These results validate the annual pattern of banding in fin-ray sections of fish up to 18 years of age. One of the consequences of not establishing and following age determination criteria is illustrated by the change in age composition due to misidentification of the first few annuli. A change in age composition has implications for stock estimates, such as mortality rates, and harvest strategies. For example, overfishing could result from underestimated mortality rates if fishery managers used a strategy that set fishing mortality equal to natural mortality. It is recommended that age determination criteria be routinely assessed to provide reliable age estimates.

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Lingcod (*Ophiodon elongatus*) are distributed off the west coast of North America in the nearshore waters from Baja, California, to the Shumigan Islands, Alaska. They have been an important component of the commercial fishery off British Columbia since the mid-1880s and a favorite target of recreational anglers since the 1950s. They inhabit nearshore waters and are commonly found along the bottom at depths ranging from 3 to 400 m; however most are found in rocky areas 10 to 100 m. Growth during the first years of life is rapid and up to age 2 it is similar for males and females, both reaching an average length of 45 cm. After age 2, females grow faster than males and the growth of males tapers off at about age 8, whereas females continue to grow rapidly until about age 12–14. For waters off the west coast of Canada, the maximum age recorded for lingcod has been 14 years for males and 20 years for females. Females reach lengths in excess of 100 cm, whereas males rarely exceed lengths of 90 cm.

The fin-ray method for age determination has been used for a wide range of freshwater and marine species such as sturgeon (*Acipenser* spp.), Pacific salmon (*Oncorhynchus* spp.), brown trout (*Salmo trutta*), whiter sucker (*Catostomus commersoni*), lake whitefish (*Coregonus clupeaformis*), channel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*), ide (*Leuciscus idus*), tuna (*Thunnus* spp.), rudd (*Scardinius erythropthalmus*), chub (*Squalis cephalus*), roach (*Rutilus rutilus*), bream (*Abramis brama*), and yellow perch (*Perca fluviatilus*) (Beamish 1981). However, in only a few instances have there been attempts in recent years to validate age estimates with the fin-ray method (Rien and Breamesderfer, 1994; Rossiter et al., 1995; Stevenson and Secour, 2000) despite the caution posed by Beamish and McFarlane (1983) that validation of age determination techniques is difficult, albeit critical.

Beamish and Chilton (1977) developed a method of aging lingcod that used thin sections of fin rays combined with mean annular diameter measurements to locate the position of the first and second annuli. This technique allowed estimates of mortality rates, growth rates, and fecundity to be determined for each age group. These rates strongly influenced assessment and management approaches for lingcod. Preliminary validation of the method was accomplished by using a mark-recapture experiment in conjunction with injections of oxytetracycline (OTC) (Cass and Beamish, 1983). However, this preliminary validation of the method was based on only four recoveries. A second experiment was initiated in 1982 to complete the validation of the fin-ray method for lingcod. Our report presents the results of this second mark-recapture study combined with OTC injections.

During the course of analyzing lingcod age data in the early 1990s, it became apparent that the annular diameter measurement criteria suggested by Beamish and Chilton (1977) were no longer being followed. Resulting parameter estimates based on these age estimates had changed. This resulted in a misunderstanding of growth, mortality, and age at maturity, which had implications for development of management strategies for lingcod. We therefore also report on a re-examination of the measurement

criteria proposed by Beamish and Chilton (1977) and show that accurate ages for lingcod can be produced by using the fin-ray method combined with mean annular diameter measurements.

Methods

During 14–27 July 1982, a major tag and release program was conducted on the commercial fishing grounds, La Perouse Bank (48′45°N; 125′55°W), off southwest Vancouver Island. The primary focus of this tagging program was for validation of age determination. Methods and survey design are outlined in Cass et al. (1983). Prior to processing, all fish were anesthetized with tricaine methane sulfonate (MS222). We measured their fork length (mm) and inserted an individually numbered Floy FD68 anchor tag into the connective tissue just below the anterior base of the first dorsal fin.

We injected the tagged fish with a 25 mg/kg body-weight dosage of oxytetracycline into the interperitoneal cavity (McFarlane and Beamish, 1987). Of the injected fish, a random subsample was kept in 3000-L holding tanks for 48 hours to assess short-term mortality due to the OTC injection, then released. We released a random subsample of tagged fish without OTC injections as a control group to assess long-term (6-month) mortality due to OTC injection by comparing recapture rates for the control fish with those for the OTC-injected fish.

Tagged lingcod were recovered by the commercial fishery off the southwest coast of Vancouver Island. Because the first annuli after the OTC mark would form during the winter of 1982–83, only tagged lingcod recovered after 1 January 1983 were used in the validation of age determination. Returned whole fish were measured for fork length (mm), and a portion of the second dorsal fin was removed for age determination according to Beamish and Chilton (1977). Sectioned fin rays were illuminated with ultraviolet light to fluoresce the OTC mark and with regular light to identify annuli.

All recovered lingcod were aged in June and August of 1987. In October 1987, a change in personnel led to an unknowing change in the criteria for determining the first few annuli. Chilton and Beamish (1982) suggested that known average diameter of the first and second annuli could be used to estimate their location. The first and second annuli can be difficult to determine when resorption of the inner portion occurs or if the center becomes obscured by irregular-shaped deposits of opaque material. In addition, the presence of checks can also make it difficult to identify the first two annuli. In all cases, an estimate of the position of the third annulus can be made by measuring the diameter of the first and second annuli from juvenile fish or fish where these annuli are visible (Chilton and Beamish, 1982). By 1988, the primary reader was no longer using the Chilton and Beamish (1982) criteria. The recovered lingcod were re-aged after this change in criteria and second ages (using these criteria) were compared with original ages (not using these criteria) to assess bias. We compared age compositions of a subsample of the lingcod with the two age estimates to illustrate the consequences of not using the Chilton and Beamish (1982) criteria.

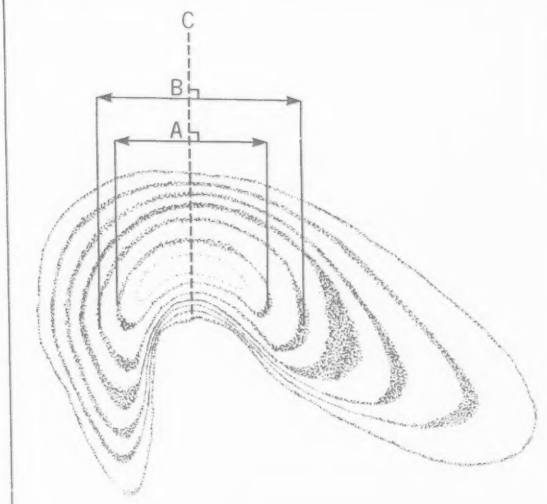

Figure 1

Schematic representation of section of fin ray for measurements of first (**A**) and second (**B**) annular diameters. Measurements should be made along axes perpendicular to the axis (**C**) of the inner groove of the fin ray.

Juvenile lingcod (less than 48 cm) were captured in 1987 in the recreational fishery in the Strait of Georgia. Fork lengths were measured (nearest mm) and dorsal fin rays were sampled during the creel survey program. These juveniles would not likely have resorbed fin-ray centers. The fin rays were sectioned (Beamish and Chilton, 1977), and illuminated with regular light. The diameter to the first and second annuli were measured with a micrometer eyepiece under 40× magnification to the nearest micrometer (μm). Diameter measurements were made along an axis perpendicular to the inner groove of the fin ray (Fig. 1). If an annuli appeared as a thick zone, the range from the beginning to the end of the zone was measured and the median used as the diameter measurement.

Results

A total of 7429 lingcod were tagged and released during July 1982. Of these, 6946 were injected with OTC and 483 were not injected but were released as the control group for assessing long-term (6-month) mortality due to OTC injection. A total of 188 lingcod were held for 48 hours after being tagged and injected with OTC. No mortality occurred prior to release. Within the first six months (i.e. by 31 December 1982) 1442 lingcod were recovered. During this period, the return rates of OTC and non-OTC–injected lingcod were similar (19.5% and 18.4%, respectively) indicating no long-term mortality from OTC injection.

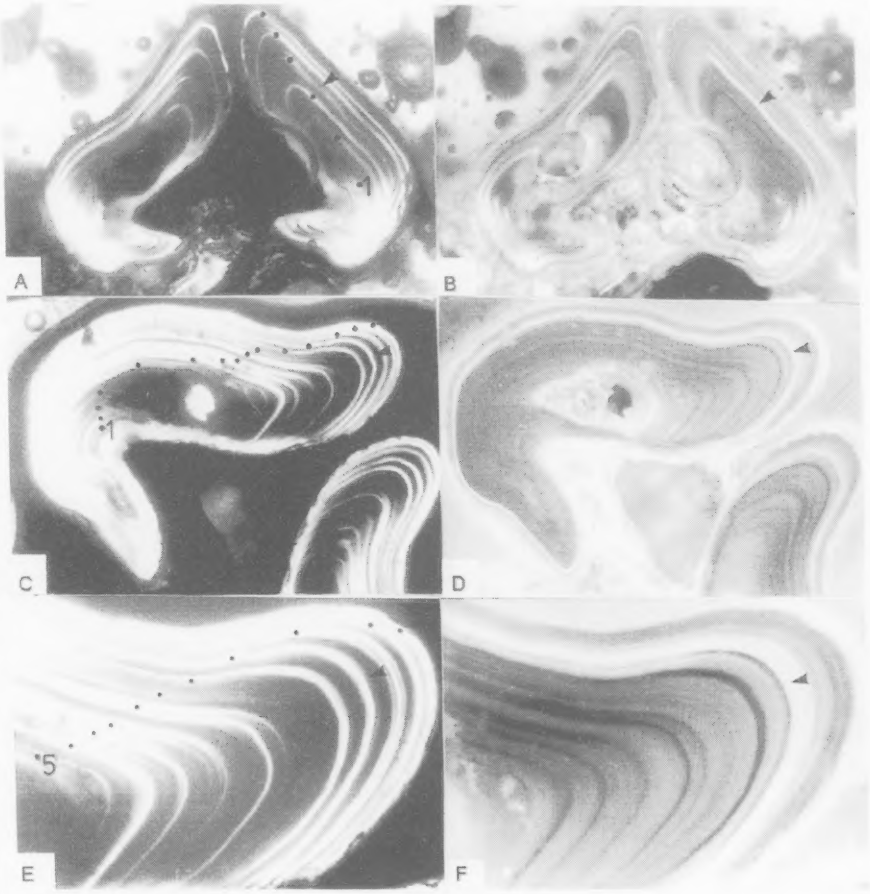

Figure 2

Annuli are indicated by dots, OTC (oxytetracycline) mark indicated by arrow. A, C, E were photographed by using white light and B, D, F were photographed by using ultraviolet light. (**A** and **B**) Fish numbered B8223905 at liberty for 2 years, with OTC mark evident after fourth annulus. Total age was 6+. (**C** and **D**) Fish numbered B8227766 at liberty for 3 years, with OTC mark evident after twelfth annulus. Total age was 15+. (**E** and **F**) Enlargements of sections C and D after the fifth annulus.

Between 1983 and 1986, 1002 tagged lingcod were recovered. From these, 460 lingcod fin rays were obtained for age determination validation: 348 recovered in 1983, 65 recovered in 1984, 43 recovered in 1985, and 4 recovered in 1986. The number of observed annuli after the OTC marks corresponded well to the number of expected annuli based on the number of years at liberty (Table 1). Lingcod recovered in 1983 were expected to have one annulus after the OTC mark and approximately 94% of the samples were estimated to have one annulus. Lingcod recovered in 1984, 1985, and 1986 had estimated annuli after the OTC mark that corresponded to the expected number of annuli in 92%, 91%, and 75% of the samples, respectively.

In most cases, the presence of the OTC mark was easily discernible and appeared as a crisp distinct line. Annuli following the mark were also clearly visible. Fish numbered B8223905 was recovered in July 1984 and aged at 6 years. The OTC mark formed just beyond the fourth annulus (Fig. 2A) and was obvious under ultraviolet light (Fig. 2B). After the OTC mark, two complete annual growth zones were present corresponding to the two years at liberty. Each annual growth zone consisted of an opaque (summer) and a translucent (winter) zone. The OTC mark at the beginning of the summer opaque zone (Fig. 2A) indicated that growth had just begun prior to tagging and injection in July.

Fish numbered B8227766 was recovered in July 1985 and aged at 15 years. As with fish B8223905, the OTC mark was also formed at the beginning of an opaque zone but was a broad band covering the whole summer zone

Table 1

The number of estimated annuli after the OTC mark versus the expected annuli based on years at large. Numbers in bold indicate those samples in which the number of observed annuli equaled the number of expected annuli.

	Expected annuli			
Estimated annuli	1	2	3	4
0	3	1	—	—
1	**328**	3	—	—
2	16	**60**	—	—
3	1	1	**39**	1
4	—	—	4	**3**

(Fig. 2C) and clearly visible with ultraviolet light (Fig. 2D). This fin ray had three annual growth zones after the OTC mark, each comprising opaque and translucent zones (Fig. 2C). An annual growth zone can contain several distinct translucent zones, i.e. checks. In this example, the tenth labelled annulus did not appear to be continuous and could, therefore, be interpreted as a check (Fig. 1, E and F). Although this area likely contained checks, the thickness and prominence of the labelled translucent zone around most of the fin-ray section (Fig. 2C) indicated that it was the edge of the tenth annulus.

Fish numbered B8224635 was recovered in July 1985 and was estimated to be 9 years old (Fig. 3). Unlike the previous two examples, the first two annuli were difficult to determine because of resorption in the central portion of the fin ray (Fig. 3). We used the diameter measurement criteria of Chilton and Beamish (1982) to locate the first and second annuli.

The 46 juvenile lingcod available from the creel survey in 1987 were used for first and second annular measurements. The mean first annular diameter was 0.40 mm (SD=0.07) and the mean second annular diameter was 0.63 mm (SD=0.11). With one exception, there was no overlap between the measured diameter for the first annulus and those measured for the second annulus and the two means were significantly different (t-test, t=17.46, df=86, P<0.0001). The mean annular diameter did not vary across the range of fork lengths observed (Fig. 4).

Ages determined by using average diameter for the location of first and second annuli measured in the late-1980s were generally one year older than ages determined without using a measurement to locate the first two annuli (Fig. 5). There appeared to be no trend related to age. However, across all ages, the unmeasured estimates ranged from three years younger to three years older than estimates made with the measurement criteria (Fig. 5). This result is likely due to the exclusion of annuli that cannot be recognized or due to the inclusion of checks, mistaken as annuli, in some fish. There was no difference in the number of annuli estimated after the OTC mark. The one-year aging bias is illustrated in the age composition

Figure 3

Fish numbered B8224635 had resorbed (R) central portion of the fin ray, making identification of the first two annuli (arrows) difficult. Subsequent annuli are indicated by dots. Total age was 9+.

for the lingcod recovered in 1983 (n=341). The age composition for the ages determined with the measurement criteria had a mode of 6 years, whereas the age composition for ages determined without the measurement criteria had a mode of 5 years (Fig. 6). For all of the samples, the estimated ages ranged from three to 18 years.

Discussion

Accurate age determination is essential for proper stock assessment and fisheries management. Although aging inaccuracies of one year might not be critical in long-lived species (e.g. 80-year-old rockfish), they can have serious stock assessment implications for shorter-lived species, such as lingcod. This is likely true because shorter-lived species typically have only four or five year classes that dominant the fishery. It is therefore important to develop accurate methods for age determination and to validate those methods. The fin-ray method first described by Beamish and Chilton (1977) is an accurate method for age determination of lingcod. Annular growth is represented by an opaque (summer growth) and a translucent (winter growth) zone and the edge of the annulus is assigned to the translucent zone (Beamish and Chilton, 1977). Using

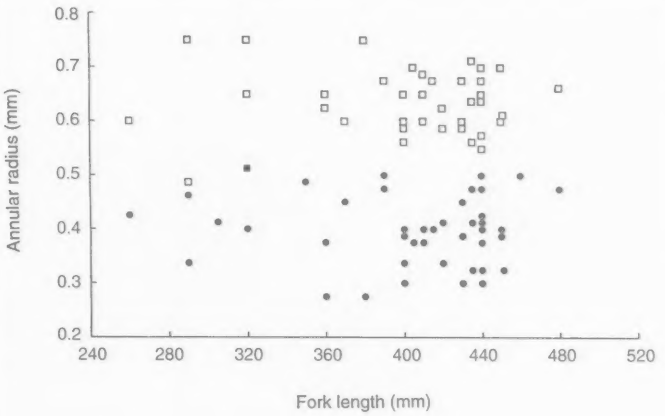

Figure 4

The mean diameter of the first (circles) and the second (squares) annuli are 0.40 mm and 0.62 mm, respectively. The mean diameters do not show a change in value across the fork lengths observed.

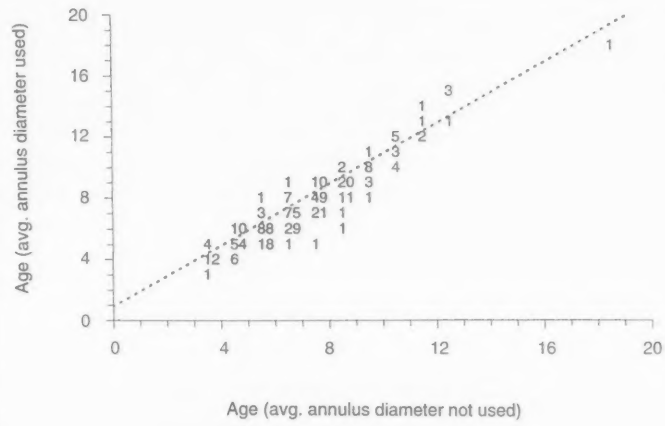

Figure 5

For all recovered lingcod, the ages determined when average annulus diameter measurements were used to estimate the location of the first and second annuli were approximately 1 year greater than ages determined when average annulus diameter measurements were not used (i.e. intercept on regression line=1). Because many data points overlapped, numbers indicate the number of observations at each point.

this method in our validation study, we found that in most cases the number of annuli estimated after an OTC mark corresponded to the number of expected annuli based on years at liberty.

Lingcod, and other species, resorb the central portion of their fin rays, making the identification of the first few annuli difficult in older fish (Beamish and Chilton, 1977). In order to obtain the most accurate ages, mean annular diameter measurements should be used routinely to locate the first and second annuli. We found no significant overlap between the first and second annular diameter measured in juvenile lingcod. It is therefore a useful criterion for

age determination and can be used for lingcod inhabiting waters off the coast of British Columbia. The fin-ray method for age determination is routinely applied to other species (Beamish, 1981) and we suggest investigators examine similar criteria to ensure the identification of the first few annuli.

Consistent use of mean annular diameter for age determination of lingcod will enable the best estimation of ages. We have illustrated that when the established criteria are not applied to identify the first few annuli, systematic underaging of approximately one year occurs—a serious consequence for stock assessment. For example, underestimates of age would overestimate mortality rates. If managers set fishing mortality equal to natural mortality (i.e. $F=M$) to estimate fishery yields, then overfishing would result (Tyler et al., 1989). If recruitment were estimated from a catch-at-age analysis, erroneous age composition and overestimates of mortality would lead to gross overestimates of recruitment (Tyler et al., 1989).

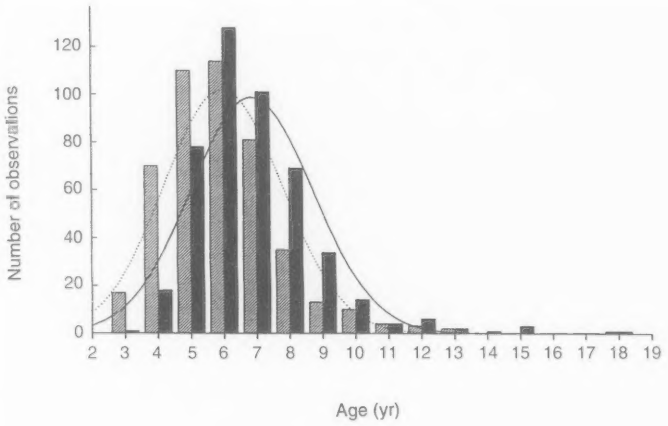

Figure 6

Age composition for the 341 lingcod recovered in 1983 when no measurements were used to estimate location of first and second annuli (striped bars) and when average measurements were used to estimate their location (solid bars).

Recently, rapid and major shifts in marine ecosystems have been shown to have consequences for the biology and behavior of a large number of fish species (Beamish et al., 2000). These shifts can occur within a year; therefore it might be expected that growth of some species could change rapidly because of environmental effects. Examination of growth time series would likely indicate a change coincident with environmental changes. However, we have illustrated that a rapid change in a growth time series can also be the result of the change in aging criteria. Therefore, for age determination to be valid and effective in fishery and ecosystem research, the criteria used should be routinely reviewed and verified.

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The behavior, growth, and survival of witch flounder (*Glyptocephalus cynoglossus*) larvae in relation to prey availability: adaptations to an extended larval period

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Abstract—Foraging behavior and prey abundance are significant factors determining the survival success of fish during the larval stage. Witch flounder (*Glyptocephalus cynoglossus*) are reported to have the longest pelagic stage of any northwest Atlantic flatfish. We used laboratory experiments to investigate the behavior and performance of witch larvae in relation to prey availability during this important life history stage. In one experiment, larvae were reared at a range of prey densities (2000, 4000, and 8000 prey per liter) and their growth and survival were monitored for 12 weeks after hatching. In a second experiment the foraging behavior of larvae was recorded during feeding trials at a range of prey densities (250, 500, 1000, 2000, 4000, 8000, and 16,000 prey per liter) during weeks 2–8 after hatching. The larval search strategy for prey appeared to change from one that was saltatory to one that was cruising, and the foraging behavior was not strongly affected by variation in prey availability. The growth rate was rapid (0.53 mm/d) and was unaffected by changes in prey density as was survival. Witch flounder larvae likely have low prey requirements compared with yellowtail flounder and Atlantic cod reared under similar laboratory conditions. The ability to forage effectively when prey is abundant or scarce and the low prey requirements of this species may be an adaptive response to the extended larval period.

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The effects of prey abundance on the behavior, growth, and survival of witch flounder (*Glyptocephalus cynoglossus*, Linnaeus) larvae were examined by using laboratory experiments. Witch flounder is an interesting study organism because it displays a life history characteristic that is very different from other Pleuronectiformes. Most flatfish undergo metamorphosis at a small size and early age (see Miller et al., 1991; Osse and Van den Boogaart, 1997). Little information is available on witch flounder larval biology and ecology, and on precise length and age data at metamorphosis for witch flounder are lacking in the literature. However, field evidence indicates that witch flounder has the longest pelagic stage of any Northwest Atlantic flatfish and undergoes metamorphosis at a relatively large size (Scott and Scott, 1988). Because of their long larval period, witch flounder larvae will likely be exposed to temporal variations in prey abundance at sea. Larvae are likely able to cope with large fluctuations in prey availability and may not be as susceptible to mismatches in prey abundance or starvation in relation to other species. Unfortunately, no information is available concerning the performance of witch flounder larvae in relation to the availability of prey in the wild. Therefore, we used laboratory experiments to gain an understanding of the early biology of this species. The objective of our study was to determine the growth, survival and foraging response of witch flounder larvae to differences in prey abundance.

Materials and methods

Eggs and milt of adult witch flounder in spawning condition were collected aboard a commercial fishing vessel in the Gulf of Maine. The eggs from approximately 10 females were fertilized with milt from an equal number of males. Fertilized eggs were shipped on ice by courier to the Ocean Sciences Centre of Memorial University of Newfoundland in Logy Bay, Newfoundland. Upon arrival, the eggs were stocked in 250-L cylindroconical upwelling incubators at 12°C. Larvae hatched over a one-day period, seven days after fertilization (day 0 of the experiment). At this point, 10 larvae were sampled for morphometric measurements (defined below).

Two experiments were designed to obtain the most information possible from the limited number of larvae available. In experiment 1, larvae were reared at a range of prey densities and their growth and survival were monitored. In experiment 2, larvae were exposed to a range of prey densities and their behavior was recorded.

We recognize that designing ecologically relevant laboratory experiments is difficult (Suthers, 2000). Our rearing conditions were chosen on the basis of established techniques for other north Atlantic marine fish larvae that have resulted in high growth and survival (Puvanendran and Brown, 1999; Rabe and Brown, 2000). Although these rearing conditions do not precisely mimic a natural environment, they do enable valid comparisons of experimental

results with those for other species. One can then use this comparative evidence to make speculations concerning observations from the field. We used cultured zooplankton as live prey for the larvae. Although this is not the natural prey for larval witch flounder, it offers desirable characteristics necessary for experimentation, such as uniform shape, size, and swimming speed and although prey densities used in our experiments were typically higher than averages reported from the field (Myers et al., 1994), these levels were necessary in the laboratory to promote growth and survival (see Houde, 1978; Puvanendran and Brown, 1999).

Experiment 1: Growth and survival

Six 33-L rectangular black tanks were used for the rearing experiment. All tanks were kept in a water bath to minimize temperature fluctuations and were supplied with filtered (25 µm) seawater. Each tank was fitted with two air stones that provided light aeration to promote a homogeneous distribution of prey. The light level at the water surface was 200 lux and continuous lighting (24 h) was used. The temperature ranged from 10° to 15°C (mean 12.6°C).

On day zero, newly hatched larvae were transferred to each experimental tank to achieve a stocking density of six larvae per liter. Three replicated feeding treatments were chosen: 2000, 4000, and 8000 prey per liter. Previous results in our laboratory for other northwest Atlantic species suggested that this range of prey densities was sufficient to observe the effects of prey density on larval growth and survival (Puvanendran and Brown, 1999; Laurel et al., in press).

Rotifers (*Brachionus plicatilis*) enriched with culture selco (INVE, Belgium) or *Artemia franciscana* nauplii enriched with DHA selco (INVE, Belgium) or Algamac (BioMarine, Hawthorne, CA), were used as prey for the larvae. Prey densities were determined by sampling 5-mL aliquots from different depths within the tanks (below surface, mid-depth, and above bottom) and were adjusted as needed to maintain nominal densities three times a day (around 10 AM, 4 PM, and 10 PM). Microalgae (*Isochrysis* and *Nannochloropsis*) were added to the experimental tanks prior to each feeding.

At week 5, the larvae were transferred to larger 65-L circular tanks because they had grown too large for the smaller tanks; all other protocols remained unchanged. The rearing experiment was stopped at week 12. At this point most larvae had begun eye migration but were still pelagic.

Data collection Larvae were sampled weekly for standard length (SL, measured from tip of snout to posterior end of notochord). For weeks 0–3, larvae were measured to the nearest 0.1 mm with a dissecting microscope. Standard length was measured to the nearest 1 mm after week 3. On weeks 1, 5, 8, and 12, five larvae were lethally sampled (killed by an overdose of MS-222) from each tank for determination of SL, body height (BH, myotome height posterior to anus), and dry weight (DW). These sampled larvae were kept in beakers on ice and measured immediately after death to prevent shrinkage from dehydration. Larvae were rinsed in 3% ammonium formate, placed on preweighed aluminum foils (weighed to nearest 0.001 mg), dried at 55°C for at least 48 hours, and reweighed. For all other weeks, the SL of ten live larvae per tank was measured.

The absolute growth rate was calculated according to the equation

$$G = (L_t - L_0)/t,$$

and the length-specific growth rate (SGR) was calculated according to the equation

$$SGR = (\ln(L_t) - \ln(L_0)/t) \times 100,$$

where L_t = the mean final length (mm);
L_0 = the mean initial length; and
t = the period of growth (days; Busacker et al., 1990).

All tanks were examined for mortalities twice daily from day 14. Dead larvae decomposed too quickly to be observed prior to this time. At the end of the experiment, the number of surviving larvae in each tank was recorded.

Data analysis For each growth measurement (SL, BH, DW), a mean value was calculated for each replicate and this value was used in the analysis. Growth measurements were analyzed by treatment using a model I analysis of covariance (ANCOVA, Zar, 1999) with age of the larvae (weeks after hatching) as the covariate (α=0.05). Dry weight data was logarithmically transformed to satisfy the assumptions of the ANCOVA. A one-way analysis of variance (ANOVA) was used to test for differences in survival at the end of the experiment.

Experiment 2: Behavior

Larvae were stocked into a 250-L cylindroconical upwelling tank on day zero. This tank served as a general rearing tank for larvae used for behavioral observations. The light was continuous (24 h) at an intensity of 200 lux at the surface. Preliminary results with witch flounder larvae showed that this light intensity and light regime resulted in good growth and survival (Rabe, 1999). The temperature ranged from 4–14°C (mean 12.4°C) during the 8-week experiment.

Feeding began on day 1 after hatching. Rotifers or *Artemia* nauplii, or both, were used as prey for the larvae and were enriched with commercial products as described previously. Larvae were fed three times daily at 4000 prey per liter (prey/L). The prey density in the rearing tank ranged from 0–4,000 prey/L throughout the day. Microalgae (*Isochrysis* and *Nannochloropsis*) were also added twice daily.

Data collection Behavioral observations were conducted every 3–4 days from week 2 to 8 beginning on day 8. The prey densities used in the feeding trials were 250, 500, 1000, 2000, 4000, 8000, and 16,000 prey/L. Prior to the

Table 1

Definition of the modal action patterns (MAPs) observed in developing witch flounder larvae, after Barlow (1968).

MAP	Definition
Locomotory MAPs	
Swim:	Forward movement of the larva through the water column resulting from undulations of the caudal region.
Turn:	A rapid lateral bend initiated by the head results in rotating the body approximately 180°.
Nondirected MAPs	
Pause:	Larva is motionless (similar to "non-swimming" of Munk, 1995).
Sink:	Larva is motionless and descends through the water column, often head first.
Shake:	Rapid lateral undulations of the entire larval body.
Foraging MAPs	
Orient:	The head movement towards a prey item (similar to "orientation" of Brown and Colgan, 1985).
Fixate:	The larva is stationary and bends its caudal region into an "S" shape position, typically follows orient MAP (Braum, 1978).
Lunge:	The larva moves towards prey from the fixate position in an attempt to capture prey (Braum, 1978).

first daily feeding, larvae were arbitrarily selected from the rearing tank and placed in 2-L glass bowls containing the appropriate density of prey. For each treatment, a total of ten larvae were serially observed for two minutes each by using the focal animal technique (Altman, 1974).

After all larvae had been observed, they were returned to the general rearing tank. The daily order of observations on prey density treatments was varied systematically over the study period. The light intensity was 200 lux during observations. On observation days, standard length was measured on 12 live larvae from the rearing tank.

Larval behaviors were categorized into modal action patterns (MAPs, Barlow, 1968). During observations, the frequency and duration of the following MAPs were recorded with an event recorder: swim, turn, pause, shake, sink, orientation, fixation, and lunge. Individual MAPs are grouped into three classes: locomotory, nondirected, and foraging (Table 1).

Data analysis The effects of prey density and larval size (mm standard length) on MAP frequency and duration were analyzed with a model III ANCOVA (Zar, 1999), with size as the covariate (α=0.05; the fixed factor was prey density and size was random). Both size and age will influence the larval response to prey density. We chose to focus on the effect of size (as a surrogate for structural characteristics), rather than age (or experience) because this factor likely has a greater effect on the foraging response to prey density during this early life history stage.

The response variable represents the mean value for the 10 individual larvae per prey density for each observation day. Means for each treatment were weighted by the inverse of the standard deviation (SD) around that mean in the ANCOVA. In cases where the SD for a treatment was zero, the mean SD for that MAP (for all prey density-size combinations) was used to weight the mean for that treatment.

A linear model was used to describe the MAPs. For the turn, pause, sink and shake duration analyses, only data for the size range prior to the near decrease or disappearance of that MAP were used, in order to satisfy the assumptions of equal variance of the ANCOVA. For the swim duration analysis, only data for the size range prior to the larvae spending most (90%) of their time swimming were used. Most behavioral response variables were logarithmically transformed to satisfy model assumptions. Plots of residuals and predicted values were examined for heteroscedasticity and normality for each test, and ANCOVA assumptions were satisfied.

The orientation MAP frequency data could not be easily fitted to a linear or polynomial equation and were analyzed differently. An ANOVA was used to determine the effects of prey density on orientation frequency within the size range where orientation frequency was variable between treatments (10.5–20.8 mm). A Tukey test was then used to determine which treatment means differed (α=0.05).

We compared the lunge frequency of early (≤2 weeks) and late (≥6 weeks) stage witch flounder larvae to that of yellowtail flounder (*Pleuronectes ferrugineus*) and Atlantic cod (*Gadus morhua*) to examine differences in prey consumption rate between species. Yellowtail flounder were observed at 8000 prey/L because this prey density promotes good growth and survival for this species (Rabe and Brown, 2000). Similarly, the data used for Atlantic cod were taken from the behavioral observations conducted at 4000 prey/L presented in Puvanendran and Brown (1999). The 4000 prey/L level was the prey density that optimized growth and survival in that experiment.

Results

Experiment 1: Growth and survival

At hatching, the mean standard length of larvae was 5.62 mm (±0.12 mm SE). The standard length, dry weight, and body height of larvae did not differ between prey density

Table 2

Summary of ANOVA and ANCOVA results for growth and foraging response variables of witch flounder larvae at different prey densities (no. of prey per liter). An ANOVA was used for orient frequency within the size range 10.5–20.8 mm; for all other response variables an ANCOVA was used. Age (weeks after hatching) was used as the covariate for growth response variables, whereas size (mm SL) was used for behavioral response variables. (* denotes a significant difference at α=0.05).

MAP	Source	df	*F*	*P*
Standard length (mm)	Age	1	7041.4	<0.001*
	Prey density	2	0.85	>0.25
	Age × prey density	2	0.75	>0.25
	Error	66		
Dry weight (mg)	Age	1	2640.4	<0.001*
	Prey density	2	0.06	>0.5
	Age × prey density	2	0.21	>0.5
	Error	18		
Body height (mm)	Age	1	582.1	<0.001*
	Prey density	2	0.03	>0.5
	Age × prey density	2	0.28	>0.5
	Error	18		
Orientation frequency	Prey density	6	4.71	<0.01*
	Error	42		
Fixate frequency	Size	1	6.94	<0.05*
	Prey density	6	1.29	>0.25
	Size × prey density	6	0.52	>0.5
	Error	84		
Lunge frequency	Size	1	15.8	<0.001*
	Prey density	6	1.35	>0.25
	Size × prey density	6	0.62	>0.5
	Error	84		

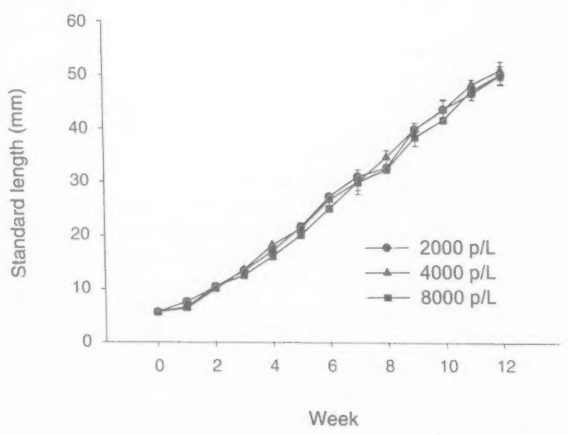

Figure 1

Mean standard length (mm) of witch flounder larvae reared at different prey densities (no. of prey per liter) over age (weeks after hatching). Values are means of the means for each replicate ±SE.

treatments (Table 2, Figs. 1 and 2). Larvae began to increase in body height around the mean size of 15 mm (Fig. 2B). The average absolute growth rate from week 0 to week 12 for all treatments was 0.53 mm/d.. The average specific growth rate (SGR) from week 0–6 was 3.68%/d and from week 0–12 was 2.61%/d.

The survival results were not corrected for fish sampled lethally (20 per tank). The survival in all treatments was similar over the course of the experiment (Table 3) and was unaffected by prey density at week 12 (ANOVA; $F_{2,3}$=2.75, P=0.210).

Experiment 2: Behavior

Larval behavior was characterized by a shift from nondirected MAPs to locomotory activities between the mean sizes of 10.5–16.2 mm (Fig. 3, A and B). The increase in locomotory activities was due to an increase in the total time spent swimming (Fig. 3A). The average duration of a swim MAP increased from 3.2 to 86.3 seconds over the study, whereas the frequency of swimming decreased significantly (Table 4). A turn MAP was used during the early stage, disappearing by the time larvae reached

10.5 mm (Fig. 3A). Pause and sink MAPs decreased when larvae reached 16.2 mm, whereas a shake MAP stopped altogether (Fig. 3B). The durations of the locomotory and nondirected MAPs changed with size (Fig. 3, A and B) but were not significantly affected by prey density (Table 4). At the end of the study there was a slight increase in pause MAPs and a concomitant decrease in the duration of swim MAPs, the result of some larvae settling during the observation periods.

Throughout the study, larvae spent between 2% and 10% of the total time observed performing foraging activities. The variation in total time spent foraging was largely due to variation in orientation duration. The duration of the fixate and lunge MAPs (<2% of total time per MAP) was relatively constant over the observation periods, whereas the duration of the orientation MAP ranged from 1% to 7% of total time (Fig. 3C).

The frequencies of the foraging behaviors were highly variable and many larvae did not forage during the observation periods. The frequency of the orientation MAP changed throughout the study period, peaking between the mean sizes of 10.5 and 20.8 mm (Fig. 4A). Within this size range, orientation frequency increased with increasing prey density. The effect of prey density on orientation frequency was significant (Table 2); the orientation frequency of larvae at 250 prey/L was significantly lower than that of larvae at 2000–16,000 prey/L within the 10.5–20.8 mm size interval (Tukey test). After 20.8 mm, the frequency of orientation was low for all sizes and treatments.

The frequencies of fixate and lunge MAPs varied from 0 to 4 per two-min observation. Larvae at higher prey densities tended to perform more fixate and lunge MAPs compared with larvae at lower prey densities (Fig. 4, B and C), although this trend was not statistically significant (Table 2). The frequencies of fixate and lunge MAPs increased significantly with increasing larval length (Fig. 4, B and C, Table 2). The average lunge frequency of early- and late-stage witch flounder larvae was lower than that of both yellowtail flounder and Atlantic cod larvae (Fig. 5).

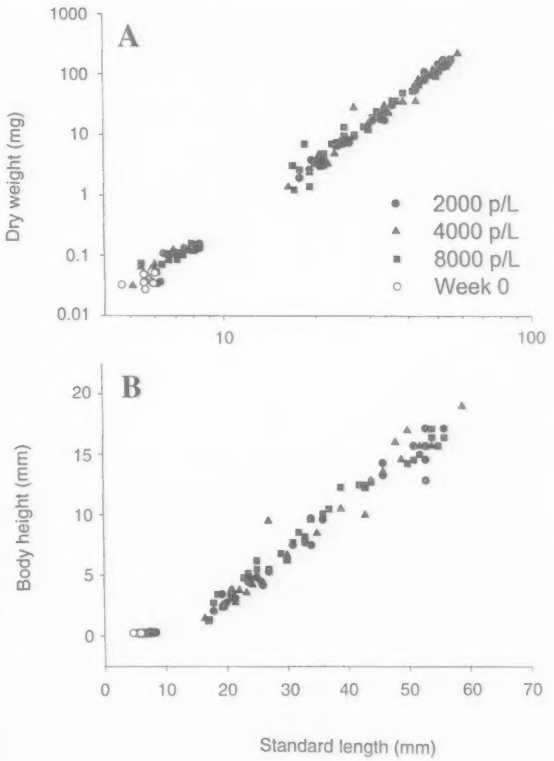

Figure 2

Relationship between standard length (mm) and (**A**) dry weight (mg; note logarithmic axis) and (**B**) body height (mm) for individual witch flounder larvae reared at different prey densities (no. of prey per liter). Symbols represent individual larvae.

Discussion

Witch flounder larvae grew and survived in all treatments used in our study. Our experiment is the first to examine the early growth and behavior of witch flounder larvae in relation to prey availability. Larval performance can be influenced by many factors other than prey density, including temperature (Hunter, 1981), light, (Batty, 1987; Puvanendran and Brown, 1998), prey type (Drost, 1987), and turbulence (MacKenzie and Kiørboe 1995; Browman, 1996). We used our results 1) to describe the early growth and ontogeny of the foraging behavior in this species and 2) as a preliminary step towards understanding the behavioral ecology of witch flounder larvae.

Table 3

Percentage of witch flounder larvae reared at different prey densities (±SE) that survived over the experiment. Larvae sampled dead were not included in calculations.

	Prey density (no. of prey per liter)		
	2000	4000	8000
Week 2	36.15 (1.28)	30.26 (6.15)	38.20 (4.36)
Week 5	28.72 (1.54)	25.13 (5.13)	31.03 (3.85)
Week 12	14.10 (2.31)	4.62 (0.51)	8.97 (4.36)

Witch flounder grew and survived equally well at each of the prey densities tested. Although the range of prey densities used in the rearing experiment (2000–8000 prey/L) was not exhaustive, these prey densities have resulted in informative differences in growth and survival for other

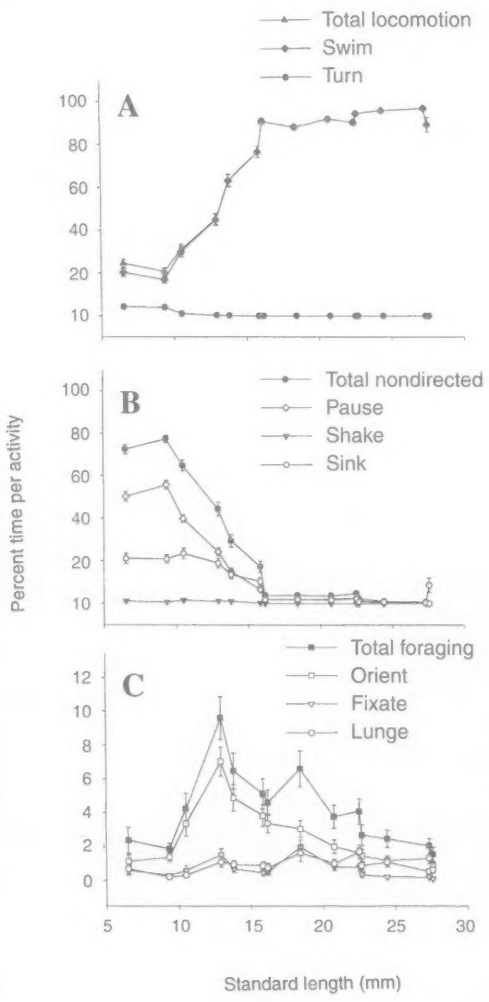

Figure 3

Mean proportion (%) of time witch flounder larvae spent performing individual (**A**) locomotory, (**B**) nondirected, and (**C**) foraging MAPs over standard length (mm) during two-minute observation periods. Values are means (n=70 larvae per length) ±SE.

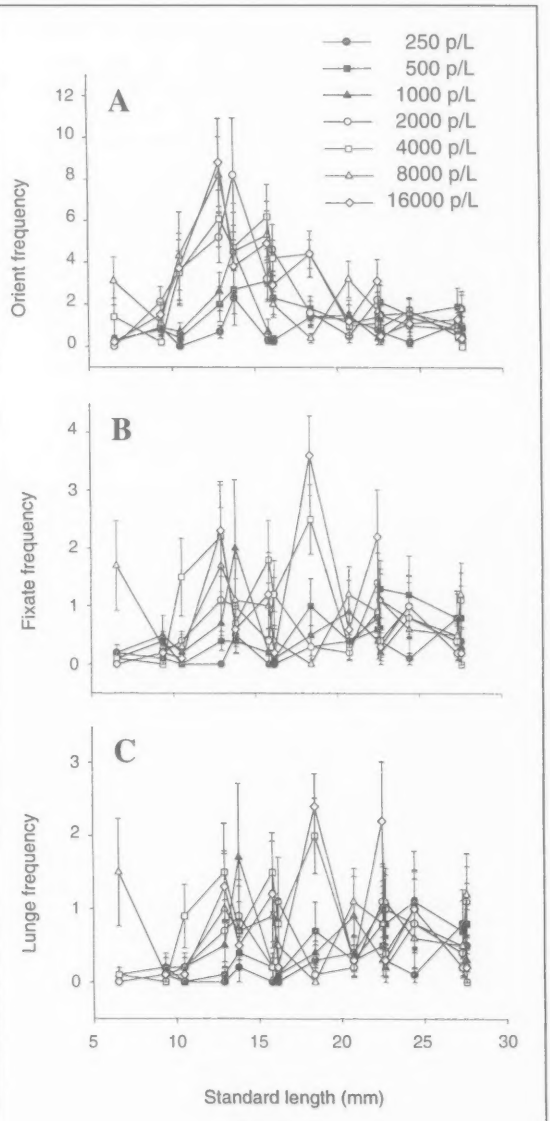

Figure 4

Frequency of (**A**) orient, (**B**) fixate, and (**C**) lunge MAPs of witch flounder larvae at different prey densities (no. of prey per liter) during two-minute observation periods over standard length (mm). Values are means (n=10) ±SE.

North Atlantic marine fish larvae, such as Atlantic cod (Puvanendran and Brown, 1999) and redfish (*Sebastes* sp.; Laurel et al., in press) reared under similar laboratory conditions. Therefore, we anticipated that this range of prey densities would be adequate to detect differences in growth and survival of witch flounder in relation to prey density. Furthermore, as shown in experiment 2, the lunge frequency of witch flounder was not significantly affected by prey availability, which would be expected if low prey densities (<2000 prey/L) were to reduce consumption and subsequent growth and survival.

Ontogeny of behavior

Witch flounder search strategy for prey is interesting because it appeared to change from a saltatory to a cruise strategy (see O'Brien et al., 1990; Browman and O'Brien, 1992) during the study period. When larvae were less than 10 mm, foraging included many turns and brief periods of swimming that served as repositioning acts. By the time larvae reached an average size of 16.2 mm, most of their

Table 4

Summary of ANCOVA results for locomotory and nondirected MAPs of witch flounder larvae at different prey densities (no. of prey per liter). Each model was run until the larval size indicated in parentheses to satisfy model assumptions. (* denotes a significant difference at α=0.05).

MAP	Source	df	*F*	*P*
Swim frequency (20.8 mm)	Size	1	32.1	<0.001*
	Prey density	6	0.78	>0.25
	Size × prey density	6	0.42	>0.5
	Error	49		
Swim duration (20.8 mm)	Size	1	251.4	<0.001*
	Prey density	6	1.76	>0.25
	Size × prey density	6	0.17	>0.5
	Error	49		
Turn duration (13.8 mm)	Size	1	56.5	<0.001*
	Prey density	6	1.34	>0.25
	Size × prey density	6	0.82	>0.5
	Error	21		
Pause duration (20.8 mm)	Size	1	88.4	<0.001*
	Prey density	6	1.82	>0.25
	Size × prey density	6	0.26	>0.5
	Error	49		
Sink duration (18.4 mm)	Size	1	79.8	<0.001*
	Prey density	6	0.54	>0.5
	Size × prey density	6	1.35	>0.5
	Error	42		
Shake duration (16.2 mm)	Size	1	13.0	<0.001
	Prey density	6	1.13	>0.25
	Size × prey density	6	0.25	>0.5
	Error	35		

time was spent swimming, which was typically interrupted only by foraging events. During the mean size interval of 10.5–16.2 mm, the turn and shake MAPs disappeared and the frequency of pause and sink MAPs decreased. These behavioral changes were likely related to the increase in larval body height, accompanied by a substantial increase in finfold height that occurs during this time.

The nature of the nondirected MAPs shake, sink, and pause is not straightforward. Sinking has been reported in other species, such as the snapper (*Pagrus auratus*) and, like the pause MAP, has been interpreted as a resting behavior. In snapper, it occurs in yolksac larvae and in feeding larvae during night-time periods of inactivity (Pankhurst et al., 1991). Sinking is typically observed only in the early stages of other species such as the black sea bream, *Acanthopagrus schlegeli* (Fukuhara, 1987). However, Kawamura and Ishida (1985) noted that sinking occurs in both yolksac larvae and larger feeding larvae of the flounder *Paralichthys olivaceus* immediately after attacking a prey item. Observations of sinking in later-stage witch flounder larvae were not related to feeding events; the persistence of these behaviors in witch flounder was likely the result of some smaller, slower-growing individuals having been included in our observations.

Witch flounder, like other species (Holling, 1965; Houde and Schekter, 1980; Werner and Blaxter, 1980; Puvanendran and Brown, 1999), demonstrated increased foraging behavior with prey density. However, the orient MAP was the only foraging MAP statistically affected by prey density. The change in orientation frequency with size is interesting because this MAP was affected only by prey density within a limited size range. The initial low frequency of orient MAPs followed by an increase associated with greater larval size can be explained by changes in swimming speeds and encounter rates (Mittelbach, 1981). However, the decrease in orient frequency across treatments later in the study period is puzzling because larger larvae are generally competent swimmers (Rosenthal and Hempel, 1971; Laurence, 1972; Houde and Schekter, 1980) and are expected to exhibit a high prey encounter rate. This decrease in orient frequency may be due to improved foraging ability associated with greater visual acuity. Miller et al. (1993) showed that the visual angle—the smallest angle at which a stimulus may subtend the eye and remain resolvable (Neave, 1984)—decreases during the development of three species of fish larvae. Thus, the eye develops such that larvae can likely detect prey items in their periphery without turning the head and orienting themselves toward them.

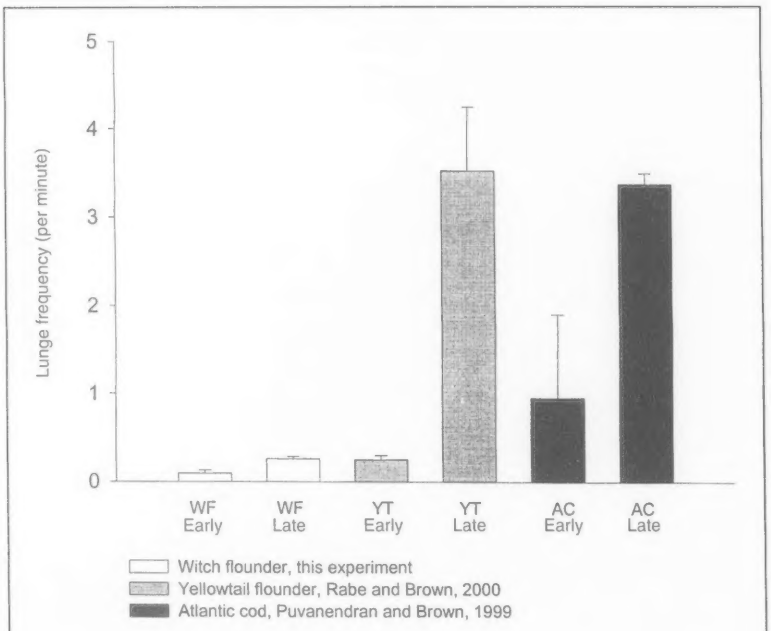

Figure 5

Average lunge frequency per minute for early (≤2 weeks) and late stage (≥6 weeks) witch flounder (WF), yellowtail flounder (YT), and Atlantic cod larvae (AT). See text for details.

Behavioral ecology

The main finding of our study was that witch flounder are not affected by changes in prey availability in the same manner as other species of larvae observed under similar laboratory conditions. The typical pattern among fish larvae—that they increase their foraging behavior and prey consumption rate with increased prey density (Houde and Schekter, 1980)—was supported by our study. However, the results are unusual in that the effects of prey density on foraging behavior were not as strong as results that have been reported for other species.

The ecological implications of these results can be illustrated by a comparison of the growth and behavior of witch flounder to other northwest Atlantic species observed under similar laboratory conditions. In rearing experiments on Atlantic cod larvae, Puvanendran and Brown (1999) found that cod have specific requirements for high prey densities. Larval survival, growth rate (0.20 mm/d), and condition were highest when larvae were reared at a prey density of 4000 prey/L. Furthermore, in the same experiment, the lunge frequency (an indicator of consumption rate) of cod larvae increased from nearly 1 to 3.5 prey items per minute over the six-week study period (Fig. 5; Puvanendran and Brown, 1999). A marked increase in lunge frequency with age is also seen in yellowtail flounder, another north Atlantic pleuronectiform, raised under similar conditions (Fig. 5; Rabe and Brown, 2000). In that study, the growth rate of yellowtail flounder was 0.34 mm/d.

Not only is the foraging behavior of witch flounder relatively unaffected by variation in prey density, but its lunge frequency is much lower, suggesting that it may have a lower consumption rate and therefore lower prey requirements compared with those of other species. The lack of a significant effect of prey density on the foraging of witch flounder is not solely a result of the larger larval size of this species. Redfish (*Sebastes*) are relatively large at extrusion (6–8.9 mm; Penny and Evans, 1985) and their foraging behavior is affected by variations in prey availability (Laurel et al., in press).

It is remarkable that the growth rate of witch flounder is faster than that of cod and yellowtail flounder, especially given the possible lower prey consumption rate of witch flounder. Two potential mechanisms that may explain this phenomenon are high assimilation efficiency and low metabolic requirements. Witch flounder are relatively large at hatching and for most of the study period were larger than other species of similar age. This size difference alone implies that its digestive system is larger, more developed, and more efficient (Govoni et al., 1986; Klumpp and von Westernhagen, 1986). Both yellowtail flounder and cod are more active and swim faster than witch flounder. Higher activity imparts a greater need for prey, which results in

larvae being more susceptible to starvation in the absence of prey (Hunter, 1981).

The ecological significance of the lack of an effect of prey density on foraging, growth, and survival found in our study may be a reflection of the life history of witch flounder. This species has an extended larval period and is committed to being in the water column much longer than other species (Bigelow and Schroeder, 1953). During this extended larval period, witch flounder larvae will likely encounter periods of high or low plankton availability, or both. The abundance of zooplankton prey for fish larvae can vary over four orders of magnitude during the year, typically reaching a peak in the warmer months and decreasing dramatically in the winter (Myers et al., 1994). Therefore, witch flounder larvae must be able to cope with this variation in prey availability to survive. This condition requires a different strategy from that of other marine fish species that have shorter larval periods and that likely rely on a "match" of spawning with plankton production to promote larval survival (Cushing, 1972). In the scope of the life history evolution of witch flounder, the long larval period and large size at metamorphosis may be a strategy to cope with intense postsettlement, size-dependent competition.

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Lack of divergence between 16S mtDNA sequences of the swimming crabs *Callinectes bocourti* and *C. maracaiboensis* (Brachyura: Portunidae) from Venezuela*

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Abstract–Lake Maracaibo, a large Venezuelan estuarine lagoon, is reportedly inhabited by three species of the genus *Callinectes* Stimpson, 1860 that are important to local fisheries: *C. sapidus* Rathbun, 1896, *C. bocourti* A. Milne Edwards, 1879, and *C. maracaiboensis* Taissoun, 1969. *Callinectes maracaiboensis*, originally described from Lake Maracaibo and assumed endemic to those waters, has recently been reported from other Caribbean localities and Brazil. However, because characters separating it from the morphologically similar *C. bocourti* are noted to be vague, we have compared these species and several congeners by molecular methods. Among our specimens from Lake Maracaibo and other parts of the Venezuelan coast, those assignable to *C. bocourti* and *C. maracaiboensis* on the basis of putatively diagnostic characters in coloration and structural characteristics do not differ in their 16S mtDNA sequences. These molecular results and our re-examination of supposed morphological differences between these species suggest that *C. maracaiboensis* is a junior synonym of *C. bocourti*, which varies markedly in minor features of coloration and structural characteristics. Genetic relationships of this species to other species of swimming crabs of the genus *Callinectes* are also explored and presented as phylogenetic trees.

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Swimming crabs of the genus *Callinectes* Stimpson, 1860 are widely distributed throughout the American tropics and subtropics, where many species are exploited commercially (Norse, 1977; Williams, 1984). Members of this genus play key trophic roles in coastal habitats that range from sandy-mud bottoms to seagrass meadows (Arnold, 1984; Orth and van Montfrans, 1987; Wilson et al., 1987; Lin, 1991). Of the nine species of *Callinectes* from the tropical western Atlantic, seven have been reported from western Venezuela (Rodríguez, 1980; Williams, 1984; Carmona-Suárez and Conde, 1996). However, knowledge of these Venezuelan populations remains inadequate, despite the importance of several species in both large-scale commercial harvests and artisanal fisheries of coastal villages in the area (Oesterling and Petrocci, 1995; Ferrer-Montaño, 1997; Conde and Rodríguez, 1999). Persistent difficulty in identifying mixed samples of these species hampers understanding of their distribution, abundance, and population dynamics, which are essential to fishery-management decisions. In particular, consistent morphological distinction of the commonly encountered *C. bocourti* from *C. maracaiboensis* cannot be achieved with confidence.

Initially described as endemic from Lake Maracaibo, *C. maracaiboensis* has been reported from other Venezuelan waters, as well as from sites in Jamaica, Curaçao, Colombia, and Brazil (Norse, 1977; Carmona-Suárez and Conde, 1996; Sankarankutty et al., 1999). Although detailed descriptions have been provided by Taissoun (1969, 1972) and Williams (1974) to diagnose *C. maracaiboensis*, the same authors have repeatedly emphasized its resemblance to *C. bocourti*. Morphological distinction of the two species is based upon postulated defining characters of the frontal teeth, direction and form of

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the anterolateral spines, convexity of the carapace, surface granulation patterns, and varied morphometric measurements (Taissoun 1969, 1972). However, neither these characters nor additional ones proposed by other authors (Williams, 1974; Rodríguez, 1980) have proven to be of consistent use in separating the species. Carapacial color and granulation vary widely, and the outer frontal teeth of many specimens cannot be classified as a definitive triangular (*C. maracaiboensis*) or obtuse (*C. bocourti*) shape. These concerns have led some authors to report only possible co-occurrence of *C. maracaiboensis* in their samples of *C. bocourti*, as in records from Puerto Rico (Buchanan and Stoner, 1988).

Recently, molecular markers have proven particularly valuable in decapod crustacean systematics. In addition to reconstructing phylogenetic relationships, molecular sequences are of value in recognizing questionable species separations (e.g. Geller et al., 1997; Sarver et al., 1998; Schneider-Broussard et al., 1998; Schubart et al., 1998; Schubart et al., in press). In our study, we used sequences of a mitochondrial gene (16S rRNA) to examine genetic differentiation between the morphologically similar species *C. bocourti* and *C. maracaiboensis* and to compare these sequences to those of other swimming crabs of the genus *Callinectes* in a phylogenetic analysis.

Materials and methods

Swimming crabs were caught by hand in shallows of the Golfete de Cuare (68°37′W, 10°06′N, State of Falcón, Venezuela) in November–December 1998 and March 1999, and in the shallows of El Moján, Lago de Maracaibo (71°39′W, 10°58′N, State of Zulia, Venezuela) in October 1999. Crabs were transported to the Instituto Venezolano de Investigaciones Científicas, Caracas, in liquid nitrogen and then stored in a –70°C freezer. Two walking legs and one swimming leg were separated from each specimen, preserved individually in 85% ethanol, and shipped to the University of Louisiana, Lafayette (U.S.A.), for molecular analyses.

Specimens were assigned to *Callinectes bocourti* or *C. maracaiboensis* according to morphological characters provided by Taissoun (1969, 1972), Williams (1974, 1978), Fischer (1978), and Rodríguez (1980). Eight specimens of *C. bocourti* and six of *C. maracaiboensis* from Venezuela were used for the molecular analysis. In addition, a short fragment (~300 base pairs) of 16S mtDNA was obtained with an internal primer for one formalin-preserved specimen of *C. bocourti* from Colombia. These voucher specimens, lacking limbs (which had been removed for analysis), were cataloged in collections of the Laboratorio de Ecología y Genética de Poblaciones, Instituto Venezolano de Investigaciones Científicas (IVIC), and in the University of Louisiana Zoological Collection (Table 1). For outgroup comparisons, specimens of *C. sapidus*, *C. ornatus* Ordway, 1863, *C. danae* Smith, 1869, and *Portunus ordwayi* (Stimpson, 1860) were sequenced (Table 1).

Genomic DNA was isolated from the muscle tissue of one walking leg with a phenol-chloroform extraction (Kocher et al., 1989). Selective amplification of a 585-basepair (bp) product (547 bp excluding primer regions) from the mitochondrial 16S rRNA gene was carried out by a polymerase-chain-reaction (PCR) (35–40 cycles: 1 min at 94°C, 1 min at 55°C, 2 min at 72°C (denaturing, annealing, and extension temperatures, respectively) with primers 16Sar (5′-CGCCTGTTTATCAAAAACAT-3′), 16Sbr (5′-CCGGTCTGAACTCAGATCACGT-3′), 1472 (5′-AGATAGAAACCAACCTGG-3′), and 16L15 (5′-GACGATAAGACCCTATAAAGCTT-3′) (for references to primers see Schubart et al., 2000). 16L15 is an internal primer and, in combination with 16Sbr, was used for partial amplification of the formalin-preserved specimen. PCR products were purified with Microcon-100® filters (Millipore Corp., Bedford, MA) and sequenced with the ABI BigDye® Terminator Mix (PE Biosystems, Wellesley, MA) in an ABI Prism 310 Genetic Analyzer® (Applied Biosystems, Foster City, CA). Sequences were manually aligned with the multisequence-editing program ESEE (Cabot and Beckenbach, 1989). New sequences were accessioned to the European Molecular Biology Laboratory (EMBL) genomic library (see Table 1). Sequences available online (by electronic database accession numbers that follow) were obtained for "*Callinectes sapidus*" (U75267), *C. ornatus* (U75268), *C. similis* (U75269), and *C. sapidus* (AJ130813).

Sequence divergence was analyzed by using Kimura 2-parameter distances, UPGMA cluster analysis, and neighbor-joining (NJ) distance analysis (Saitou and Nei, 1987) with the program MEGA (Kumar et al. 1993). Statistical significance of groups within inferred trees was evaluated by the interior branch method (Rzhetsky and Nei, 1992). As a second phylogenetic method, a maximum parsimony (MP) analysis was carried out with a heuristic search and random sequence addition (tree-bisection and reconnection as branch-swapping option) and by omission of gaps with the program PAUP (Swofford, 1993). Significance levels were evaluated with the same software and the bootstrap method with 2000 replicates.

Results

Sequencing of 15 specimens of *Callinectes bocourti* and *C. maracaiboensis* from Golfete de Cuare and El Moján (both Venezuela) and the Río Sinú estuary (Colombia) revealed the existence of seven different haplotypes. Two haplotypes (ht-1 and ht-5) clearly predominated (together 64.3%) and were found in both species (ht-1 in two *Callinectes bocourti* and three *C. maracaiboensis* from Golfete de Cuare, ht-5 in three *C. bocourti* and one *C. maracaiboensis* from El Moján). The other five haplotypes (ht-2, ht-3, ht-4, ht-6, ht-7) each differed from ht-1 or ht-5 in not more than two positions and were found in only single individuals (Fig. 1, Table 2). Consequently there is not a single diagnostic molecular character in our sequenced unit of 16S mtDNA that would discriminate between the two species of swimming crabs. On the other hand, haplotype distributions differed between the two sampled populations; ht-1 is found in only the Golfete de Cuare (~550 km east of Lake Maracaibo) and ht-5 is restricted to El Moján (within Lake Maracaibo). Comparison of the

Table 1

Swimming crabs used in sequencing of 16S mtDNA and subsequent phylogenetic analyses. IVIC-LEGP = Laboratorio de Ecología y Genética de Poblaciones, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela; ULLZ = University of Louisiana Zoological Collections, Lafayette, U.S.A.; specimens bearing numbers for both collections are archived in ULLZ; EMBL = European Molecular Biology Laboratory.

Species	Locality of collection	Catalog number	EMBL accession no.
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-40 = ULLZ 4181	AJ298171
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-70	AJ298182
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-71	AJ298172
Callinectes maracaiboensis	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-72	AJ298173
Callinectes maracaiboensis	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-1	AJ298177
Callinectes maracaiboensis	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-6	AJ298183
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEPG-C-30 = ULLZ 4180	AJ298170
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-109	AJ298174
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-112	AJ298175
Callinectes bocourti	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-113	AJ298176
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-2	AJ298181
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-3	AJ298178
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-4	AJ298179
Callinectes bocourti	Venezuela: Zulia: El Moján	IVIC-LEGP-MZ-5	AJ298180
Callinectes bocourti	Colombia: Río Sinú	ULLZ 4186	AJ298169
Callinectes sapidus	Florida: Fort Pierce	ULLZ 3766	AJ298189
Callinectes sapidus	Venezuela: Zulia: El Moján	ULLZ 4188	AJ298190
Callinectes ornatus	Venezuela: Falcón: La Vela de Coro	IVIC-LEGP-LV-CO-1	AJ298187
Callinectes ornatus	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-CO-3	AJ298188
Callinectes ornatus	Brazil: Ensenada de Ubatuba	ULLZ 4178	AJ298186
Callinectes danae	Brazil: Ensenada de Ubatuba	ULLZ 4179	AJ298185
Callinectes danae	Venezuela: Falcón: Golfete de Cuare	IVIC-LEGP-C-1	AJ298184
Portunus ordwayi	Venezuela: Falcón: La Vela de Coro	IVIC-LEGP-LV-9	AJ298191

Table 2

Percent (uncorrected) genetic divergence and number of differences between 547 base pairs of 16S mtDNA among 7 haplotypes (ht) corresponding to 14 specimens of *Callinectes bocourti* and *C. maracaiboensis* from Venezuela (s: transition, v: transversion, i: indel). Catalog numbers shown below correspond to IVIC-LEGP specimens listed in Table 1.

	ht-1	ht-2	ht-3	ht-4	ht-5	ht-6	ht-7
	C-30 C-40 C-71 C-72 C-109	C-70	C-112	C-113	MZ-1 MZ-3 MZ-4 MZ-5	MZ-2	MZ-6
ht-1	—	0.18	0.18	0.18	0.18	0.55	0.18
ht-2	1v	—	0.37	0.37	0.37	0.73	0.37
ht-3	1s	1s, 1v	—	0.37	0.37	0.73	0.37
ht-4	1s	1s, 1v	2s	—	0.37	0.73	0.37
ht-5	1s	1s, 1v	2s	2s	—	0.37	0.37
ht-6	2s, 1i	2s, 1i, 1v	3s, 1i	3s, 1i	1s, 1i	—	0.73
ht-7	1s	1s,1v	2s	2s	2s	3s, 1i	—

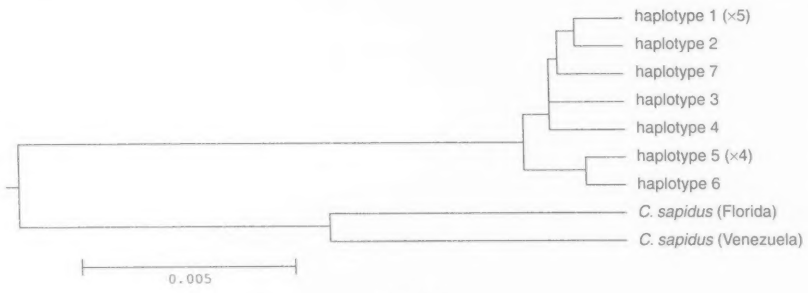

Figure 1

UPGMA (unweighted pair-group method with arithmetic averaging) analysis of Kimura 2-parameter genetic distances based on 547 base pairs of 16S mtDNA among seven haplotypes of *Callinectes maracaiboensis* and *C. bocourti* (see Table 2 for species designation) corresponding to 14 specimens from Golfete de Cuare (haplotypes 1–4) and El Moján (haplotypes 5–7) (both Venezuela) and two populations of *C. sapidus*.

294 base pairs obtained for one *Callinectes bocourti* from Colombia did not reveal any difference from ht-1, ht-3, or ht-5, suggesting that genetic homogeneity in these species can be expected in other Caribbean localities.

Comparing sequences from *C. danae*, *C. ornatus*, *C. sapidus*, and *Portunus ordwayi* with those of *C. bocourti* and *C. maracaiboensis* allowed us to postulate phylogenetic relationships among these selected portunid taxa (Fig. 2). Marked genetic differences (Table 3) characterized all intra- and interspecific separations within the genus *Callinectes*, except in pairings of *C. bocourti* and *C. maracaiboensis*.

Discussion

Comparison of 15 sequences of the 16S mtDNA of *Callinectes maracaiboensis* and *C. bocourti* from within and outside Lake Maracaibo revealed no consistent differences between these two species of swimming crabs. These results support observations from previous studies in which morphological characters have proven unreliable in separating these two species. Rather than constituting a separate species, *C. maracaiboensis* would appear from these findings to represent only a phenotypic extreme of *C. bocourti*, a species of known morphological plasticity (Williams, 1974).

We are highly confident that our sequenced samples of *C. maracaiboensis* represent the population originally assigned to that species. In addition to assuring that our samples were topotypic, we confirmed individual specimen identities by direct comparisons with deposited paratypes of Taissoun (IVIC-LEGP 425). However, we cannot make similar claims for the analyzed materials of *C. bocourti*. Although published records (Williams, 1984) indicate that *C. bocourti* is widely distributed (from Jamaica and Belize to Brazil, occasionally northward to Florida, Mississippi, and North Carolina), the type locality is southern Belize. To date, topotypic materials of *C. bocourti* have not been available to us for use in either our sequencing or morphological comparisons with Venezuelan material; such comparisons should, however, ultimately be undertaken to firmly anchor the synonymy we have proposed. Among specimens that we would presently assign to *C. bocourti* on the basis of structural features, one from the Río Sinú of Colombia shared complete identity in almost 300 base pairs of mtDNA with the most common haplotypes from Venezuela. This finding at the very least suggests a large overall range for materials that we refer to as *C. bocourti* and a relative homogeneity among forms that we have assigned to this name on the basis of structural characteristics, even over large geographic distances.

The reported phylogenetic relationships and genetic distances (Fig. 1) reflect a low level of genetic divergence among the seven haplotypes of *C. maracaiboensis* and *C. bocourti*, especially when compared with sequences of two populations of *C. sapidus*. The tree resulting from the phylogenetic analysis (Fig. 2) also suggests that *C. maracaiboensis* and *C. bocourti* are closer to *C. sapidus* than to *C. ornatus*, *C. similis*, and *C. danae*, which is in accord with preliminary findings of Norse and Fox-Norse (1979). However, our results must also be regarded as preliminary because other species of *Callinectes* should be sequenced before we can claim full understanding of sister-species relationships. Published studies to date that have attempted to separate questionable brachyuran species on the basis of 16S mtDNA have consistently reported at least a few nucleotide differences that are diagnostic for the species in question (Geller et al., 1997; Schneider-Broussard et al., 1998; Schubart et al. 1998; Schubart et al., 2000). Only the Mediterranean species *Brachynotus sexdentatus* and *B. gemmellari* may constitute an exception to this pattern because they are identical in the sequenced fragment of 16S mtDNA (Schubart et al., in press).

Comparison of our sequences with those from a previous study (Geller et al., 1997) revealed important, apparently intraspecific differences between specimens of *C. ornatus* as well as between specimens of *C. sapidus*. Genetic

Table 3

Percent genetic divergence (uncorrected; excluding indels) between 553 base pairs of 16S mtDNA among species of *Callinectes* and the outgroup *Portunus ordwayi*, as used for the phylogeny in Figure 2 (1=*C. similis* (U75269); 2="*C. sapidus*" (U75267); 3=*C. sapidus* (FL); 4=*C. sapidus* (Venezuela); 5=*C. bocourti* (ht-1); 6=*C. bocourti* (ht-5); 7=*C. danae* (Venezuela); 8=*C. danae* (Brazil); 9=*C. ornatus* (U75268); 10=*C. ornatus* (Venezuela CO-1); 11=*C. ornatus* (Venezuela CO-3); 12=*C. ornatus* (Brazil); 13=*P. ordwayi*).

	2	3	4	5	6	7	8	9	10	11	12	13
1	0.18	5.61	5.79	6.33	6.15	0.54	0.54	2.53	3.07	3.07	2.89	13.02
2		5.06	5.24	5.79	5.61	0.54	0.54	2.53	3.07	3.07	2.89	12.12
3			1.27	2.53	2.35	5.79	5.79	5.97	6.87	6.87	6.69	13.92
4				2.71	2.53	6.15	6.15	6.15	7.05	7.05	7.05	13.92
5					0.18	6.51	6.51	6.33	6.51	6.51	6.33	14.10
6						6.51	6.51	6.15	6.33	6.33	6.33	14.29
7							0.00	2.71	3.62	3.62	3.44	13.92
8								2.71	3.62	3.62	3.44	13.92
9									1.63	1.63	1.45	12.66
10										0.36	0.18	12.84
11											0.18	13.02
12												13.38

differences between Geller et al.'s (1997) specimen from North Carolina (U75268) and three specimens of *C. ornatus* from South America can be attributed to geographical distance. In the case of *C. sapidus*, however, differences clearly exceed a level that could be interpreted as ancestral polymorphism or biogeographical variation. Although our new sequence of *C. sapidus* from Florida perfectly matches one previously registered for a specimen from Louisiana (AJ130813), and both of these show limited divergence from a Venezuelan specimen, another sequence previously reported for *C. sapidus* (U75267) by Geller et al. (1997) differs markedly from the aforementioned set. The phylogeny of all 16S sequences presently available for *Callinectes* (Fig. 2) shows that the sequence reported for "*C. sapidus*" by Geller et al. (1997) instead pairs closely with *C. similis*, a grouping supported by high bootstrap values. Because no morphological voucher specimens appear to exist for this sequenced specimen,[1] we must conclude that the reported sequence was based upon a misidentified specimen of *C. similis*.

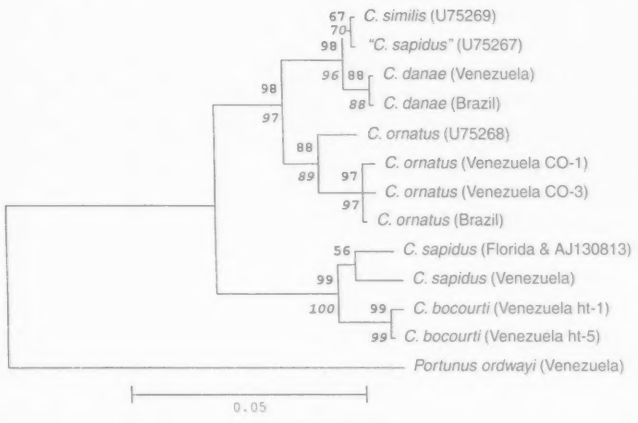

Figure 2

Phylogenetic relationships of five species of *Callinectes* based on 553 base pairs of 16S mtDNA. Tree topology is based on neighbor-joining (NJ) analysis with Kimura 2-parameter genetic distances. Sequences accessed online for *C. similis*, putative "*C. sapidus*," and *C. ornatus*, and *C. sapidus*, are indicated solely by electronic database accession numbers. Analyses for Venezuelan *C. ornatus* include specimens from adjacent localities (see Table 1); those for *C. bocourti* include haplotypes (ht) 1 and 5 (see Table 2 and Fig. 1). Confidence values ≥50 were obtained with the interior branch method for NJ distance analysis (upper values) and by maximum parsimony analysis after 2000 bootstrap replicates (lower values, in Italics).

Differences in color of some of the Brazilian specimens (see Sankarankutty et al., 1999) could indicate that two or more species might be involved in the *C. bocourti* complex, regardless of the synonymy that we have herein proposed. However, color differences should be judged with precaution, given the known high variability of this feature in *C. bocourti* (see Williams, 1984). Our recent color photography of specimens from Colombia, Venezuela, and

[1] Geller, J. 1999. Personal commun. Moss Landing Marine Laboratories, Moss Landing, CA 95039.

Florida has revealed marked variations; dorsal surfaces of the carapace of some specimens were predominantly olive green, those in other specimens were largely rust brown or rust patterned on green, and in yet others a rust pattern on a background of almost totally blue.

As a result of our molecular analyses, especially in the absence of consistent characters to distinguish *C. maracaiboensis* on the basis of color or structural characteristics, we must conclude that Lake Maracaibo and adjacent Venezuelan waters are inhabited by populations assignable to *C. sapidus* and by only one other species of the genus, *C. bocourti*. It appears that these are in turn the target species of a largely unregulated crab fishery which represented more than U.S. $6 million in exports to the United States in 1992 (Oesterling and Petrocci, 1995), even though it has declined since 1989 in probable response to overharvesting. As maximum sustainable yield has likely been achieved, these fisheries, which have been proposed for Miller's phase II management (resource-mapping, gear related regulations), should in the near future proceed to phase III (basic biological studies, long-term management) (Miller, 1999). This phase can be undertaken only with a clear understanding of the genetic entities upon which the fishery is based.

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Feeding habits, prey fields, and potential competition of young-of-the-year walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, 1994–1995

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Abstract–Diets of young-of-the-year (YOY) walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) were compared between seasons (summer and autumn), years (autumn), and allopatric and sympatric fish aggregations (autumn) in Prince William Sound (PWS), Alaska. Fish were collected principally by mid-water trawl 20 July–12 August 1995, 5–14 October 1995, and 7–13 November 1994. Prey fields were assessed from zooplankton samples in 1995.

During the summer, the principal prey of allopatric pollock and herring was small calanoids and diet overlap was high (R_0>0.76). During the autumn, diets were composed of large calanoids, larvaceans, and euphausiids. Diet overlap between sympatric species was greater in November 1994 (R_0≤0.94) than in October 1995 (R_0≤0.69). The seasonal diet shift to larger prey coincided with larger fish size and with decreased abundance and proportions of the principal zooplankter, small calanoids, and increased abundance and proportions of large calanoids and larvaceans in zooplankton tows. However, feeding decreased in autumn, compared with summer, especially for herring. Sympatric fish had higher rates of nonfeeding than allopatric fish, and subtle differences in prey selection existed between the aggregations, but sampling variation could explain these feeding differences.

The similarity in diets of YOY pollock and herring indicate the potential for competition. These species are important to commercial fisheries and as forage for marine birds and mammals. An understanding of their trophic interactions could help to explain shifts in fish community structure and bird predation. If sympatry increases as prey resources decline, competition in autumn may be particularly important in regulating populations.

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Walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) are forage fish that inhabit the northeastern Pacific Ocean rim. Both species are important components of marine bird, mammal, and fish diets, both support important commercial fisheries in the Gulf of Alaska (GOA), and historical data show dramatic variability in both their populations (Springer, 1992; Bechtol, 1997; Springer and Speckman, 1997; Anderson and Piatt, 1999). Young-of-the-year (YOY) walleye pollock and YOY Pacific herring are found at the same locations and depths during at least part of the year (Brodeur and Wilson, 1996a; Willette et al., 1997; Stokesbury et al., 2000) and both consume zooplankton as their primary prey[1,2] (Willette et al., 1997; Foy and Norcross, 1999a, 1999b). Because of these similarities and because the frequency and nature of their interactions may change as fish population structure shifts, we investigated the potential for feeding competition between these species.

Several recent studies have found that the species composition of forage fish populations in the GOA and Prince William Sound (PWS) has changed dramatically (Bechtol, 1997; Kuletz et al., 1997; Anderson and Piatt, 1999). Short-term population changes were attributed to the *Exxon Valdez* oil spill in March 1989 (Brown et al., 1996a; Kuletz, 1996; Oakley and Kuletz, 1996; Kuletz et al.,

[1] Jewett, S., and E. Debevee. 1999. Diet composition, diet overlap and size of 14 species of forage fish collected monthly in Prince William Sound, Alaska, 1994–1996. *In* Forage fish diet overlap, 1994–1996, p. 10–37. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 97163C), Auke Bay Laboratory, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, AK 99801-8626.

[2] Boldt, J. L. 1997. Condition and distribution of forage fish in Prince William Sound, Alaska. Unpubl. M.S. thesis, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11120 Glacier Hwy., Juneau, AK 99801, 155 p.

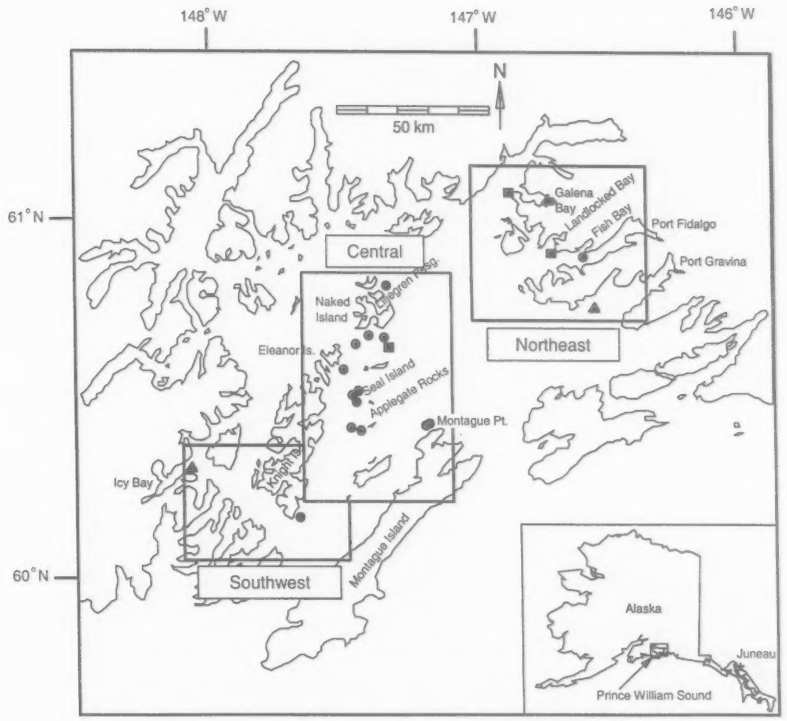

Figure 1

Sampling regions and stations for YOY walleye pollock and Pacific herring diet samples collected in Prince William Sound, Alaska. Circles: July–August 1995; squares: October 1995; triangles: November 1994.

1997), but researchers believe that long-term changes began with a climate regime shift prior to the oil spill (Bailey et al., 1995; Piatt and Anderson, 1996; Anderson and Piatt, 1999). For example, the number of walleye pollock and other demersal fish increased at the same time that the other taxa decreased (Anderson and Piatt, 1999), the PWS spawning population of Pacific herring declined by 75% by 1993 (Brown et al., 1996b), and fish biomass of PWS decreased by 50% (Piatt and Anderson, 1996). Concomitant decreases in populations of marine birds and mammals in PWS may be related to these shifts in the composition and abundance of forage fish prey (Oakley and Kuletz, 1996; Piatt and Anderson, 1996; Iverson et al., 1997). Apparently, fewer high-quality forage fish have been available, and the species composition has changed to one in which the predominant fish taxa are less energetically valuable to marine piscivores (Piatt and Anderson, 1996; Anthony and Roby, 1997; Anderson and Piatt, 1999; Payne et al., 1999). The potential for such shifts to cascade throughout marine food webs (Livingston, 1993; Springer and Speckman, 1997) is an important reason to understand the trophic interactions of forage fish.

This reports stems from the Alaska Predator Ecosystem Experiment (APEX), a multidisciplinary study that attempted to link current knowledge about the forage fish of PWS with their seabird predator populations. We describe differences in the feeding habits of YOY walleye pollock and YOY Pacific herring caught in summer and autumn in PWS and compare feeding attributes of fish caught in allopatric (single species) and sympatric (co-occurring, multispecies) aggregations in autumn to support the hypothesis that the presence of potential competitors may induce changes in feeding habits. We compare fish size, zooplankton prey fields, fish feeding habits, prey selection, food quantity, and diet overlap of these species.

Materials and methods

Field methods

Fish stomach and zooplankton samples were collected during APEX forage fish population surveys in central, northeastern, and southwestern PWS[3,4,5] (Fig. 1). In a pilot study in 1994, we sampled from 7 to 13 November aboard the Alaska Department of Fish and Game RV *Medeia*; in 1995, we sampled from 20 July to 12 August aboard the charter FV *Caravelle*, and from 5 to 14 October aboard the RV *Medeia*. Surveys were conducted offshore along a grid of parallel transects spaced at two-mile

intervals and ending as near shore as possible. Bottom depths averaged approximately 120 m (range: 25–220 m). The grid was surveyed twice in summer and once, partially, each autumn. Hydroacoustic and hydrographic profile data were collected but are presented elsewhere.[3,4,5] Where fish were detected with hydroacoustic equipment, we either interrupted the survey or returned after the transect was completed to fish with a mid-water beam trawl. The net was generally fished 20–35 minutes each trawl.[3,4] The trawl's effective mouth opening was 50 m^2, and net mesh sizes diminished from 5 cm in the wings to 1 cm in the codend. A 0.3-cm mesh liner was sewn into the codend, which terminated in a plankton bucket having 500-µm nytex mesh. In summer, beach-seine and dip-net samples occasionally supplemented the trawl catches. Subsamples of forage species (n=10 to 15 per species) were preserved in 10% buffered formalin-seawater solution on the vessels for later stomach analysis in the laboratory. We classified samples collected between 08:00 and 20:00 as "day" and those between 20:01 and 07:59 as "night."

In 1995, the zooplankton prey spectrum was assessed from dual vertical hauls taken at each station within two hours of fish catches by using conical nets that were 0.5 m in diameter and equipped with 303-µm mesh in summer and 243-µm mesh in autumn. We towed the nets from a standard depth of 20 m or to the depth at which fish were caught (or using a combination of both depths). Depth of hauls were categorized as "shallow" (≤25 m) or "deep" (>25 to <100 m). Samples were collected at both depths at seven stations in summer (from 95-1-53 to 95-1-62 and 95-1-112) and one station in autumn (95-2-7, Table 1).

Laboratory methods

After a minimum of six weeks in formalin solution, fish samples were transferred to a solution of 50% isopropanol for at least 10 days before stomach analysis was performed. Ten specimens of each species were measured (mm fork length, FL; mg wet weight), and size was used to develop age-class categories for diet samples (Smith, 1981; Paul et al., 1998a). Walleye pollock (20 to 120 mm FL) and Pacific herring (60 to 120 mm FL) were classified as YOY (age-class 0). Stomachs were excised, weighed, and their contents were removed. The weight of prey contents was recorded as the difference between full and empty stomach weights. Fish were considered to have been feeding if their stomachs contained more than a trace of food. Relative stomach fullness was recorded as integers representing empty stomachs (1), stomach containing trace contents (2), stomachs that were 25%, 50%, 75%, or 100% full (3–6), or stomachs that were distended (7). State of digestion was recorded as partially digested contents (1), mostly digested contents (2), and empty stomachs (3).

Stomach contents and zooplankton samples were identified with a binocular microscope to the highest taxonomic resolution possible and enumerated. The prey category of calanoid copepods was also segregated into "large" (≥2.5 mm total length, TL) and "small" individuals (≥2.5 mm TL). We pooled the common pelagic cyclopoid copepod *Oithona similis* with small calanoids. We subsampled all zooplankton samples and stomach samples when practical, using a Folsom splitter to achieve a minimum count of 200 of the predominant taxon. Counts were expanded and total prey weights were determined by multiplying the expanded number observed by the mean weight per taxon. Weights per taxon were obtained from data on file (specimens from zooplankton samples or fish stomachs) collected from spring through autumn of various years in southeastern Alaska or PWS (Coyle et al., 1990; Stark[6]; senior author, unpubl. data).

Analytical methods

Forage fish were considered to occur in allopatric aggregations if only one species and one age class were caught in a net haul. They were considered to be sympatric if at least two species or age classes (≥10 fish each) were caught together. For this study, we restricted analyses to YOY pollock and herring that were allopatric or that cooccurred only with each other to limit the complexity of trophic interactions; we excluded pollock and herring that were caught in other types of aggregations, such as with other species or older conspecifics. We examined the size of forage fish and their feeding attributes. Size included FL and wet weight. Feeding attributes included measures of the quantity of food consumed, measures of feeding frequency, and measures of prey composition. Food quantity was expressed as means of the total number and weight of prey (ln-transformed), stomach fullness index (rounded to nearest 25%), and prey percent body weight (%BW; ratio of wet stomach-content weight to fish body weight). Feeding frequency was measured as the percentages of feeding fish and the percentages of fish with partially or mostly digested stomach contents. Prey composition was expressed as the percent number and percent biomass of prey categories. Zooplankton density per cubic meter and numerical percent composition was calculated for species,

[3] Haldorson, L. 1995. Fish net sampling. *In* Forage fish study in Prince William Sound, Alaska, p. 55–83. *Exxon Valdez* Oil Spill Restoration Project 94163A Annual Report, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11120 Glacier Hwy., Juneau, AK 99801.

[4] Haldorson, L. J., T. C. Shirley, and K. O. Coyle. 1996. Biomass and distribution of forage species in Prince William Sound. *In* APEX project: Alaska predator ecosystem experiment in Prince William Sound and the Gulf of Alaska (D. C. Duffy, compiler), Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 95163 A–Q), Alaska Natural Heritage Program, Department of Biology, Univ. Alaska Anchorage, 707 A Street, Anchorage, AK 99501.

[5] Haldorson, L. T. Shirley, K. Coyle, and R. Thorne. 1997. Forage species studies in Prince William Sound. *In* Alaska predator ecosystem experiment in Prince William Sound and the Gulf of Alaska (D. C. Duffy, compiler). Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 96163 A–Q), Alaska Natural Heritage Program, Department of Biology, Univ. Alaska Anchorage, 707 A Street, Anchorage, AK 99501.

[6] Stark, C. 1995. Personal commun. Institute of Marine Science, Univ. Alaska Fairbanks, P. O. Box 757220, Fairbanks, AK 99775-7220.

Table 1

Mean zooplankton density and percent density (standard errors, SE, in parentheses) from pooled vertical hauls in summer (n=37 hauls, 303-μm mesh) and autumn (n=8 hauls, 243-μm mesh), and from shallow (≤ 25 m) and deep (50–100 m) hauls in Prince William Sound, 1995.

	Mean density (number of zooplankton/m^3)					
	Summer			Autumn		
Prey category	Overall	Shallow	Deep	Overall	Shallow	Deep
Barnacle larvae	<1	<1	<1	<1	<1	<1
Calanoids, large	29 (4)	37 (7)	28 (6)	204 (60)	104 (3)	176 (3)
Calanoids, small	1018 (133)	1175 (181)	550 (87)	828 (130)	685 (48)	426 (40)
Chaetognaths	4 (<1)	3 (1)	4 (1)	3 (<1)	1 (<1)	5 (<1)
Cladocera	33 (9)	25 (9)	6 (1)	0	0	0
Cyphonautes larvae	0	0	0	205 (50)	297 (7)	333 (8)
Decapod larvae	1 (<1)	2 (<1)	<1	0	<1	0
Euphausiid larvae	3 (1)	3 (1)	2 (<1)	2 (<1)	1 (<1)	2 (1)
Gastropods	60 (10)	99 (23)	36 (5)	96 (19)	141 (4)	84 (5)
Hyperiid amphipods	2 (<1)	2 (<1)	2 (<1)	1 (<1)	1 (<1)	1 (<1)
Larvaceans	14 (4)	6 (2)	8 (3)	45 (11)	50 (12)	22 (7)
Other	17 (2)	17 (3)	8 (1)	14 (2)	18 (6)	9 (1)
Total[1]	1184 (138)	1371 (191)	645 (91)	1414 (185)	1299 (64)	1064 (56)
	Percent composition					
	Summer			Autumn		
Prey category	Overall	Shallow	Deep	Overall	Shallow	Deep
Barnacle larvae	0	0	0	0	0	0
Calanoids, large	3	3	5	13	8	17
Calanoids, small	84	84	84	58	53	40
Chaetognaths	<1	<1	<1	<1	<1	<1
Cladocera	5	2	1	0	0	0
Cyphonautes larvae	0	0	0	16	23	14
Decapod larvae	0	0	0	0	0	0
Euphausiid larvae	<1	<1	<1	<1	<1	<1
Gastropods	6	7	6	7	11	8
Hyperiid amphipods	<1	<1	<1	<1	<1	<1
Larvaceans	<1	<1	<1	2	4	2
Other	2	1	1	1	1	1

[1] Numbers do not add to column totals because of rounding.

principal prey taxa, and total organisms in each vertical tow by using the expanded organism count divided by the water volume of the tow; mean values of replicate tows were used to represent each station.

Feeding selectivity of allopatric and sympatric aggregations of pollock and herring was calculated for summer and early autumn, 1995, when zooplankton were collected at the fish sampling stations. Occasionally, in summer, zooplankton samples from adjacent stations were substituted for those fishing stations without prey samples (Table 1). At stations where zooplankton was collected at two depths, the selection values presented were based on zooplankton from the depth where fish diet samples were collected. We used Strauss' linear selection index, L_0 (Strauss, 1979), a measure varying from –1 to +1, where negative values indicate no preference for the prey taxon and positive values indicate preference for the prey taxon:

$$L_0 = r_i - p_i,$$

where r_i = percentage of i^{th} prey resource in the diet; and
p_i = percentage of i^{th} prey resource in the environment.

Prey resources for selection were defined as the species, stages, and sizes of prey pooled into principal taxa.

Feeding overlap between species and within species between fish in allopatric and sympatric aggregations was described by using Horn's overlap index (Horn, 1966; Smith and Zaret, 1982; Krebs, 1989). This index minimizes bias due to changing numbers of resource categories and resource evenness. Overlap was computed at two levels: prey resources were defined at the lowest level (species, stage, and size) and at a pooled level (principal taxa). Horn's overlap index values, R_0, were expressed from 0 (no overlap) to 1 (total overlap) for predator species j and k:

$$R_0 = \frac{\sum (p_{ij} + p_{ik}) \times \ln(p_{ij} + p_{ik}) - \sum p_{ij} \times \ln p_{ij} - \sum p_{ik} \times \ln p_{ik}}{2 \times \ln 2}$$

where p_{ij} = proportion of i^{th} resource in total prey resources utilized by j^{th} species; and
p_{ik} = proportion of i^{th} resource in total prey resources utilized by k^{th} species.

We considered R_0 values >0.60 to indicate similar use of resources and R_0 >0.75 to indicate very similar use of resources.

In this report, we examined both intraspecific and interspecific differences in YOY pollock and herring and qualitatively compared data between seasons, between years (in autumn), between allopatric and sympatric aggregation types, and between sympatric species. We also compared day-night feeding frequencies and prey condition to assess principal time of day of feeding. We compared zooplankton densities and percent densities of principal taxa between seasons and between depths (shallow and deep) sampled each season. Because our data were limited and the sampling design unbalanced, we present means and standard errors without statistical tests.

Results

Zooplankton prey fields

Total zooplankton densities for all samples pooled were similar in summer and autumn of 1995, averaging approximately 1200 and 1400 organisms/m^3 (n=37 and 8, mesh=303 µm and 243 µm, respectively; Table 1, Fig. 2). Approximately 3/4 of samples from each season were collected in daylight, between 10:00 and 20:00.

Zooplankton taxa were less diverse in summer than in autumn, but small calanoids predominated in both seasons. Seasonal differences in the density and percentage contribution of a few taxa were apparent. Small calanoids made up a greater percentage of the total in summer compared with autumn (84% vs. 58%), but their density was similar in each season. Large calanoid density and percent density were both lower in summer than in autumn. Only a few other taxa contributed ≥5% to the total numbers of zooplankters in either season. In summer, these included cladocera and gastropods (primarily the pteropod *Limacina helicina*); in autumn, they included large calanoids, bryozoan cyphonautes larvae, and gastropods (Table 1, Fig. 2).

Calanoid species composition in the zooplankton also varied seasonally. Small calanoids in summer were predominantly *Pseudocalanus* spp. (75%) and *Acartia longiremis* (5%); in autumn, *Pseudocalanus* spp. was 40%, *Oithona similis*, 15%, and *A. longiremis*, 6% of total zooplankton. Large calanoids in summer were principally *Neocalanus* and *Calanus* spp. (3% of total zooplankton) and in autumn, they were *Metridia pacifica* (10%) and *Calanus* spp. (1%). Among other taxa present in both seasons, density and percent density of gastropods were similar between seasons, but larvaceans were less available in summer than in autumn. Cladocera were present only in summer and cyphonautes only in autumn. Hyperiid amphipods, euphausiid larvae, chaetognaths, and barnacle and decapod larvae were rare in both seasons (Table 1).

Zooplankton density per cubic meter varied with depth of sampling at all stations for both seasons. In summer (n=7 stations), zooplankton density was more than twice as high in shallow hauls as in deep hauls, whereas in autumn (n=1 station), it was greater in the shallow tows than in the deep tows (Table 1). Depth-related differences in the abundance of principal taxa, but not in their percentage composition, were also observed. Overall, in summer, small calanoids were twice as abundant in the more shallow tows, but they comprised similar percentages at each depth. In autumn, both density and percentage of small calanoids were greater in shallow tows than in deeper tows. Large calanoid density and percentage did not differ between depths in summer, but in autumn they were lower in shallow tows compared with deep tows. Gastropod abundance in both seasons, and cladoceran abundance in summer, were greater in shallow tows than in deep tows, but percentages did not differ with depth. Larvacean abundance and percentage abundance in either season, and the abundance and percentage abundance of cyphonautes in autumn, did not differ between depths.

The macrozooplankters, larval euphausiids and young hyperiids, were present, but rare in our plankton tows. However, euphausiids were captured in 11% of summer trawls and in 43% of autumn trawls. In summer of 1995,[4] juvenile–adult euphausiids were present in the northeastern and southwestern regions of the sound but were not caught in the central region or at any stations where fish were caught; in autumn (1994[7] and 1995[4]), they were present in trawls from all three regions but were absent from the allopatric trawl in October 1995. We have no consistent data on the presence or absence of hyperiid amphipods.

Fish catches

Catches of YOY walleye pollock and Pacific herring sampled from PWS that met our allopatric-sympatric criteria (see "Materials and methods" section) were not evenly rep-

[7] Paul, A. J. 1995. Invertebrate forage species. *In* Forage fish study in Prince William Sound, Alaska, p. 43–54. *Exxon Valdez* Oil Spill Restoration Project 94163A Annual Report, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11120 Glacier Hwy., Juneau, AK 99801.

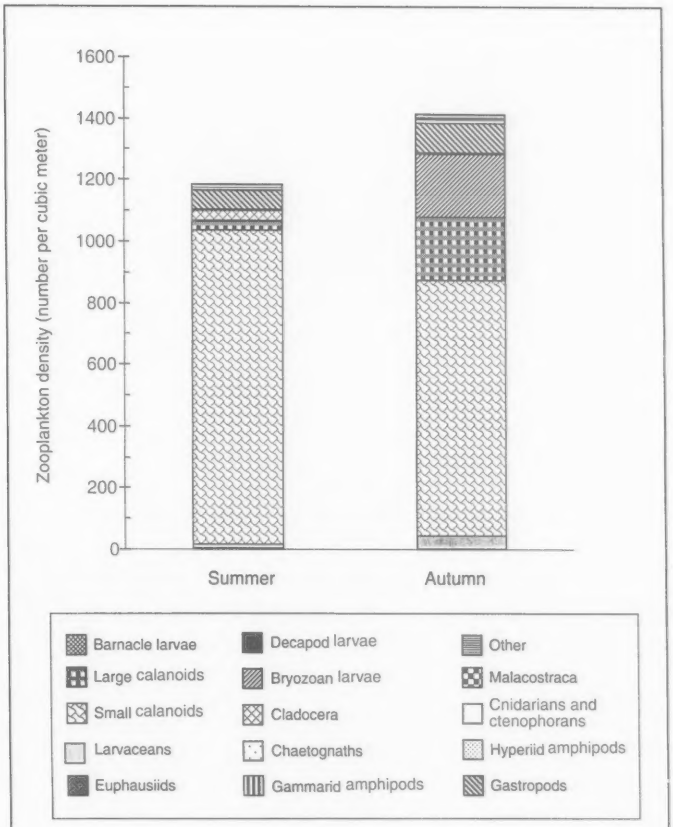

Figure 2

Seasonal zooplankton density and composition by principal taxa in Prince William Sound, Alaska 1995. Zooplankton were collected in vertical tows of conical nets having 303-µm mesh in summer and 243-mm mesh in autumn.

resented in both seasons. Only allopatric fish qualified in the summer of 1995, but both allopatric and sympatric fish qualified in the autumns of 1994 and 1995 (Table 2). In summer, 18 of the 62 trawl hauls caught sufficient samples of either species.[4] Allopatric YOY pollock were collected at 12 stations in the central region and allopatric YOY herring were collected at one central and one northeastern station in summer (Fig. 1). In autumn, 11 of the 25 trawl hauls (total 14 in November 1994,[3] 11 in October 1995[4]) caught sufficient samples of either species, and 36% of these caught sympatric fish in the northeastern region. Allopatric species were collected in different years: the allopatric pollock in November 1994 were collected in the southwestern region and the allopatric herring in October 1995 in the central region (Table 2). The remainder of pollock and herring caught did not meet our study criteria for age, number, or composition of fish per haul.

The magnitude and relative species composition of the catch varied considerably, and sampling time and depth differed between seasons. In summer, the number of pollock caught in trawls varied by two orders of magnitude between stations, from 22 to 1689 per haul (Table 2). Herring catches in the alternative gear, dip net or beach seine, were of similar magnitude. In autumn, from 14 to 4156 pollock or herring were caught per trawl haul; the catches were not evenly partitioned between the species in sympatric hauls. Similar numbers of pollock and herring were caught at some sympatric stations, whereas, at others, the number per species caught was an order of magnitude greater. In summer, approximately half the samples were collected in the morning, half in the afternoon, and only one was collected at dusk (~22:00). The pollock were caught most often at 50–80 m trawl depths ($\bar{x}$=60 m) offshore and the herring were caught at the surface and near shore. In autumn, sympatric samples were collected in darkness (approximately 22:00–23:00) at a mean depth of 30 m in bays, whereas allopatric samples were collected during daylight and in deeper water (Table 2).

Table 2

Sampling stations, catch, mean size, and feeding attributes for allopatric and sympatric YOY walleye pollock and Pacific herring (n=10 each) from locations in PWS in summer 1995 and autumns 1994–1995. Zooplankton samples were collected at most fishing stations only in 1995; asterisks indicate stations where an adjacent zooplankton haul was substituted. Standard error of the means (SE) is shown in parentheses. Abbreviations: T = trawl, D = dip net, B = beach seine, NE = northeast, C = central, SW = southwest.

Sampling period, target catch, cruise number, and gear	Region and location	Number caught	FL (mm)	% feeders	Fullness (%)	Content %BW	Total number of prey	Total weight of prey (mg)
Summer 1995								
Allopatric Pacific herring								
95-1-27D*	NE, Fish Bay, Port Fidalgo	271	76 (2.0)	100	100	3.8 (0.4)	5968.7 (604.3)	540.71 (62.8)
95-1-110B*	C, Southeast Eleanor Is.	—[1]	30 (0.3)	60	25	0.0 (0.0)	52.6 (28.6)	1.55 (0.7)
Allopatric walleye pollock								
95-1-3T	C, Applegate Rocks	74	59 (1.5)	60	25	0.7 (0.2)	171.0 (113.0)	14.25 (7.5)
95-1-5T	C, Seal Island	31	54 (1.2)	60	50	1.2 (0.3)	37.1 (14.1)	27.27 (12.0)
95-1-19T*	C, Lilhengren Passage	139	58 (1.7)	80	50	1.8 (0.5)	12.4 (3.6)	12.11 (5.0)
95-1-53-1T	C, Montague Point	—[1]	56 (1.1)	100	75	1.1 (0.1)	154.1 (27.5)	13.35 (2.4)
95-1-53-2T	C, Montague Point	694	62 (1.3)	100	75	1.2 (0.2)	261.5 (38.9)	34.84 (5.7)
95-1-54T	C, East Knight Island	1056	54 (1.6)	60	25	1.3 (0.3)	53.5 (30.2)	4.68 (2.2)
95-1-56T	C, Seal Island	1689	58 (1.8)	50	25	0.7 (0.2)	21.8 (10.5)	4.86 (2.1)
95-1-57T	C, Seal Island	386	58 (2.6)	80	50	1.4 (0.5)	45.0 (19.6)	38.59 (26.0)
95-1-58T	C, Knight-Seal Island	843	56 (2.1)	100	100	2.2 (0.4)	242.0 (84.8)	33.70 (9.6)
95-1-62-2T	C, East Eleanor Island	293	53 (1.1)	90	50	1.4 (0.2)	17.2 (4.3)	15.99 (7.8)
95-1-108T*	C, Southeast Knight Is.	22	66 (1.4)	100	100	2.7 (0.4)	2489.5 (351.1)	193.24 (24.6)
95-1-112T	C, South Naked Island	504	65 (2.8)	90	50	0.9 (0.1)	20.6 (4.5)	14.79 (5.0)
October 1995								
Allopatric Pacific herring								
95-2-5T	C, East Naked Island	89	92 (1.4)	100	50	1.4 (0.1)	385.9 (89.5)	103.36 (19.5)
Sympatric Pacific herring								
95-2-6T	NE, Galena Bay	425	84 (4.3)	90	75	1.2 (0.2)	927.1 (303.6)	58.63 (11.4)
95-2-7T	NE, Landlocked Bay	592	95 (3.3)	90	50	0.5 (0.1)	271.1 (103.7)	84.50 (62.3)
Sympatric walleye pollock								
95-2-6T	NE, Galena Bay	4156	93 (2.8)	100	100	1.2 (0.2)	78.3 (14.2)	61.26 (14.4)
95-2-7T	NE, Landlocked Bay	535	90 (2.4)	90	50	0.5 (0.1)	46.8 (15.0)	16.79 (3.9)
November 1994								
Allopatric walleye pollock								
94-2-5T	SW, Icy Bay	61	111 (1.6)	100	100	1.8 (0.4)	722.6 (156.2)	71.62 (11.4)
Sympatric Pacific herring								
94-2-6T	NE, Galena Bay	26	93 (4.1)	20	10	0.1 (0.0)	13.8 (9.0)	0.78 (0.5)
94-2-7T	NE, Port Gravina	398	97 (4.2)	70	25	0.5 (0.2)	31.8 (14.7)	25.64 (12.7)
Sympatric walleye pollock								
94-2-6T	NE, Galena Bay	145	100 (3.1)	70	50	0.5 (0.5)	18.5 (5.1)	18.29 (9.2)
94-2-7T	NE, Port Gravina	14	111 (1.7)	60	25	0.4 (0.1)	6.4 (1.9)	14.56 (7.5)

[1] Data missing.

Table 3

Seasonal fish size and feeding attributes of allopatric and sympatric YOY walleye pollock and Pacific herring from Prince William Sound, 1994–1995. Values are mean and standard error, SE, for fish pooled from stations shown in Table 1.

Species and sampling period	*n*	FL	Wet weight (g)	% non-feeders	Fullness (%)	Content % BW	Total number of prey	Total weight of prey (mg)
Walleye pollock								
Summer (allopatric)	120	58.0 (1.0)	1.1 (0.1)	19	50 (5)	1.4 (0.1)	294 (68)	34.0 (5.6)
Oct. 1995 (sympatric)	20	91.5 (2.4)	5.0 (0.3)	5	75 (10)	0.8 (0.3)	63 (11)	39.0 (8.9)
Nov. 1994	30	107.2 (2.0)	8.1 (0.3)	23	50 (10)	0.9 (0.2)	249 (80)	34.8 (7.2)
Allopatric	10	111.0 (1.6)	9.1 (0.4)	0	100 (5)	1.8 (0.4)	723 (156)	71.6 (11.4)
Sympatric	20	105.4 (2.1)	7.6 (0.5)	35	25 (10)	0.4 (0.1)	13 (3)	16.4 (5.8)
Pacific herring								
Summer (allopatric)	20	52.7 (2.4)	1.5 (0.3)	20	75 (10)	1.9 (0.5)	3011 (739)	271.1 (69.0)
Oct. 1995	30	90.2 (2.0)	6.6 (0.3)	7	50 (5)	1.0 (0.1)	528 (119)	82.2 (21.6)
Allopatric	10	91.6 (1.4)	6.8 (0.4)	0	50 (10)	1.4 (0.1)	386 (90)	103.4 (19.5)
Sympatric	20	89.5 (3.0)	6.5 (0.6)	10	75 (10)	0.9 (0.1)	599 (173)	71.6 (31.0)
Nov. 1994 (sympatric)	20	94.6 (2.4)	6.9 (0.3)	55	10 (10)	0.3 (0.1)	23 (9)	13.2 (6.8)

Size of YOY pollock and herring

Intraspecific patterns of size across seasons differed for YOY pollock and herring. Mean FLs for both species were approximately 60% longer in autumn than in summer. Herring size was similar in autumn of both years, but pollock were 14 mm longer in FL and 50% heavier in November 1994 than in October 1995 (Tables 2 and 3). Interspecific comparisons of size showed that YOY pollock were generally longer, but not heavier, than YOY herring in the same season.

Feeding habits of YOY pollock and herring

Prey composition of YOY pollock was similar to that of YOY herring in both summer and autumn (Figs. 3 and 4), but prey compositions differed between seasons. Small prey predominated in summer and larger prey in autumn, especially in terms of biomass composition.

In summer, small calanoid copepods (*Pseudocalanus* spp., *Centropages abdominalis,* and *Acartia longiremis*) dominated the diets, both numerically and in terms of prey biomass (Figs. 3 and 4). For pollock, small calanoids constituted 55% by number and 57% by weight of diet. By number, most of the remainder of summer pollock diet comprised minute invertebrate eggs (39%); by weight, the remainder was large calanoids (principally *Calanus pacificus*, *C. marshallae,* and *Metridia pacifica*), fish, hyperiid amphipods, and euphausiids (both larvae and older stages, including *Thysannoessa* sp.). Pollock also commonly consumed small amounts of other prey, such as larvaceans, gastropods, and chaetognaths. For herring, small calanoids represented proportionally more of the diet than for pollock in summer. Small calanoids made up 77% by number and 88% by weight of herring diet; they were the sole taxon consumed by the YOY herring at Eleanor Island station 110 (Table 2). Most of the rest of herring diet comprised other small prey (cladocerans, bivalve larvae, and minute invertebrate eggs), whereas decapod larvae, gastropods, hyperiids, and euphausiid larvae made minor contributions.

In autumn of both years, pollock and herring again fed on the same prey categories. Compared with summer, small calanoids composed smaller percentages of the diet (only up to 37% of prey number and 9% of prey biomass). Larvaceans and large calanoid copepods numerically dominated their diets (57–91%; Fig. 3), whereas euphausiids and large calanoids generally dominated in terms of biomass percentages (49–89%; Fig. 4). Hyperiids occasionally contributed substantial dietary biomass. Fish in all of the autumn aggregations ate a variety of sizes of juvenile–adult euphausiids (*T. raschii, T. spinifera,* and unidentifiable species), and amphipods (*Themisto pacifica*, *Primno macropa,* and *Hyperia* sp.), but not the larval stages observed in summer diets. The large calanoids consumed included the same species present in summer diets, as well as *M. okhotensis* and *Neocalanus* spp.; the small calanoids included *Pseudocalanus* spp., *Acartia longiremis*, and *Oithona similis*. Some differences in autumn diet composition existed between years. Invertebrate eggs (the majority of "other") were present less frequently in autumn diets than in summer diets. In general, the October 1995 diets included proportionally more biomass from large calanoids, whereas the November 1994 diets included proportionally more biomass from euphausiids and proportionally more numbers from larvaceans.

Diet overlap between YOY pollock and herring was high, particularly when prey species were grouped into principal taxa (Table 4). In summer, diets were very similar ($R_0 \geq 0.76$) between allopatric species in terms of numbers and weights of prey species or principal prey taxa. In autumn, interspecific diet overlap was also observed

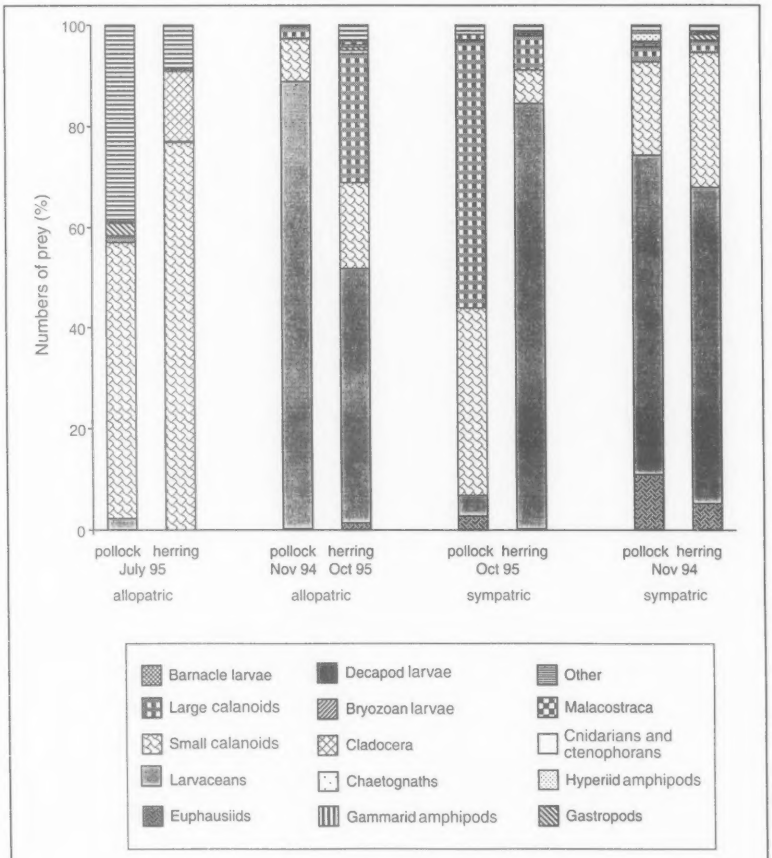

Figure 3

Percent total number of prey consumed by YOY walleye pollock and Pacific herring from sympatric and allopatric aggregations in Prince William Sound, Alaska, in July–August 1995, October 1995, and November 1994.

in terms of prey numbers and weights. Numeric overlap for the November 1994 sympatric species was observed at both stations, and the mean was approximately twice that for the October 1995 sympatric species (R_0=0.97 versus 0.43). Biomass overlap between sympatric fish occurred at three out of four autumn stations when based on principal prey taxa, but only at one station (in November) when based on prey species. Overall, diets of sympatric pollock and herring overlapped more in terms of biomass in November (R_0=0.95) than in October (R_0=0.69). We did not have autumn samples from the same year to compare allopatric diet overlap between species.

Prey selection

We noted selection from zooplankton prey resources by pollock in summer and by pollock and herring in autumn, 1995 (Fig. 5). Selection patterns were almost identical whether calculated from shallow or deep zooplankton abundances; values were always in the same direction (selection or avoidance), and their magnitude was within 10 points. Neither species selected small calanoids, the most abundant zooplankton taxon in both seasons. In summer, even though small calanoids made up >50% of YOY pollock and herring diets (Figs. 3 and 4), this taxon was avoided by pollock and was consumed in close proportion to its availability by herring (Fig. 5). Summer pollock moderately selected for large calanoids, gastropods, and larvaceans, but summer herring did not strongly select for any prey category.

Changes in zooplankton composition from summer to autumn were reflected in fish diets and prey selection. The percent density of small calanoids in the zooplankton declined by nearly 30% from summer to autumn, the percentage consumed by fish was likewise much reduced, and selection values were more strongly negative. In contrast, both large calanoids and larvaceans were more abundant in zooplankton samples in autumn than in summer and,

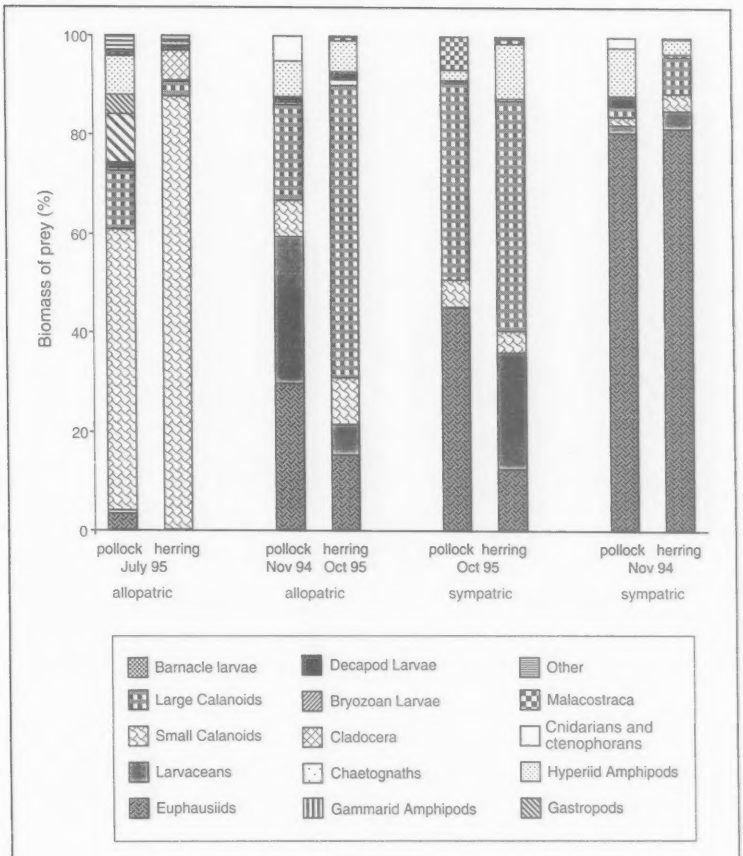

Figure 4

Percent total biomass of prey consumed by YOY walleye pollock and Pacific herring from sympatric and allopatric aggregations in Prince William Sound, Alaska, in July–August 1995, October 1995, and November 1994.

along with euphausiids and hyperiids, formed larger dietary components at that time. In autumn (October 1995), pollock strongly selected for large calanoid copepods and herring strongly selected for larvaceans (Figs. 3–5). Additional differences were noted in terms of prey frequency. In summer, pollock consumed hyperiids more frequently than they consumed euphausiids, and in autumn, both pollock and herring consumed euphausiids more frequently than hyperiids.

Seasonal feeding

Several measures of feeding in autumn indicated that YOY pollock continued to feed moderately, whereas the pattern for YOY herring showed more of a decline from summer (Tables 1 and 3). Very similar high percentages of pollock and herring had fed in summer and in October 1995 (≥80%), but in November 1994, the percentage of nonfeeding herring was greater than the percentage of feeding herring, and was more than twice the percentage of nonfeeding pollock (Table 3). For pollock, the November 1994 feeding measures were only lower than the high summer measures, whereas for herring, feeding measures in autumn of 1994 were lower than in either summer or autumn of 1995. Although pollock stomachs were at least half full in each season, herring stomach fullness declined from 75% full in summer to half full in October 1995, to only trace amounts of food in November 1994. Similarly, prey content %BW declined less between seasons for pollock than for herring. Total prey numbers and weights were generally lower for pollock than for herring in a season, but numbers of prey were highly variable and biomass of prey was relatively stable for pollock, whereas these measures showed declining trends for herring (Fig. 6, Table 3).

Digestion data and feeding-frequency data for individual fish were pooled across seasons to compare day and night feeding patterns. The condition of stomach contents

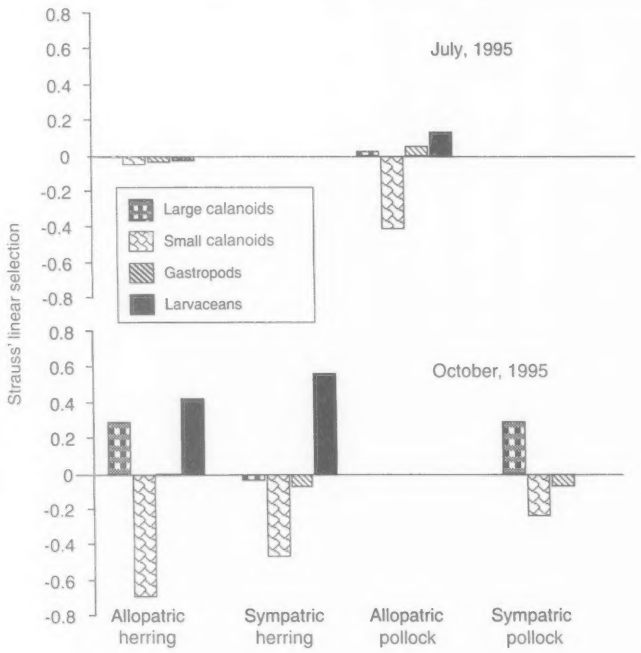

Figure 5

Strauss' selectivity index (mean) for principal prey groups of allopatric and sympatric YOY walleye pollock and Pacific herring from PWS in July–August and October 1995. Selection was not computed for November 1994, when zooplankton were not collected. Positive values indicate selection, negative values avoidance.

Table 4

Horn's overlap index values for total numbers and biomass of prey consumed by YOY walleye pollock and Pacific herring caught separately in summer and together in autumn in Prince William Sound, 1994–1995. No summer sympatric fish were available and autumn allopatric fish were not caught in the same year. Overlap greater than 0.60 indicates similar diets (see text). C = central; NE = northeast.

			Overlap in number		Overlap in biomass	
Sampling period and target catch		By prey Region	By prey species	By prey category	By prey species	category
Interspecific diet overlap						
July 1995 allopatric fish	1995	C, NE	0.79	0.82	0.76	0.83
October 1995 sympatric fish	1995–96	NE	0.16	0.22	0.44	0.64
	1995–97	NE	0.43	0.48	0.53	0.69
	Average	NE	0.31	0.43	0.55	0.69
Intraspecific diet overlap						
November 1994 sympatric fish	1994–96	NE	0.69	0.94	0.08	0.39
	1994–97	NE	0.86	0.91	0.88	0.91
	Average	NE	0.87	0.97	0.88	0.95
Allopatric-sympatric fish						
October 1995 herring			0.51	0.89	0.56	0.93
November 1994 pollock			0.87	0.91	0.56	0.73

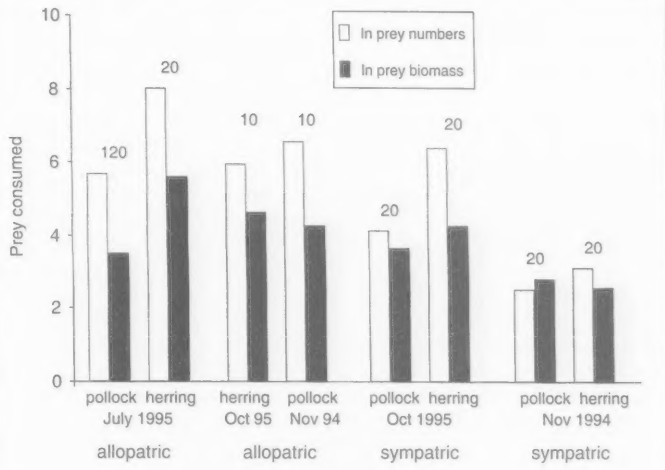

Figure 6

Total number and total biomass of prey (ln-transformed means) consumed by allopatric and sympatric YOY walleye pollock and YOY Pacific herring from Prince William Sound, Alaska, in July–August 1995, October 1995, and November 1994. Number of individuals is indicated.

by time of day was different for the two species. Pollock had greater percentages of mostly digested contents during the day than during the night (before and after 22:00). Conversely, herring had greater percentages of mostly digested contents during the night than during the day. The day-night percentages of feeding and nonfeeding fish were similar for both, however.

Comparisons between allopatric and sympatric fish aggregations

The allopatric-sympatric size pattern was different for pollock and herring. We did not pool sympatric fish from October 1995 and November 1994 for comparison with allopatric groups because of the interannual differences in size and feeding measures. For pollock in November 1994, FLs of allopatric and sympatric fish were similar, but fish were approximately 1.5 g lighter in sympatric aggregations than fish in allopatric aggregations. For herring in October 1995, the sizes of allopatric and sympatric fish were similar (Table 3).

The allopatric-sympatric feeding pattern was somewhat different for pollock than for herring in autumn. Among November 1994 pollock, the allopatric fish consistently had the highest feeding measures and the sympatric fish consistently had the lowest feeding measures (Fig. 6, Table 3). This finding coincided with slightly increased numerical percentages of larvaceans, increased gravimetric percentages of euphausiids, and increased frequencies of euphausiids, amphipods, and large calanoids in the diet of allopatric pollock compared with the diet of sympatric pollock (Figs. 3 and 4). For October 1995 herring, allopatric fish consumed the greatest prey biomass and %BW, but their prey numbers and fullness index were lower than those of sympatric fish (Fig. 6, Table 3). This finding coincided with decreased numerical percentages of larvaceans, increased numerical and gravimetric percentages of large calanoids and euphausiids, and increased frequencies of occurrence of hyperiids and euphausiids in the diet of allopatric herring compared with diet of sympatric herring (Figs. 3 and 4). Allopatric herring were more selective of large calanoids, but selection for larvaceans was similar for both groups of herring (Fig. 5). For both species, intraspecific diet overlap between allopatric and sympatric fish was extensive at the principal taxon level. In October 1995 intraspecific overlap in terms of prey biomass was 0.93 for herring, and in November 1994, intraspecific overlap in terms of prey number was 0.91 for pollock.

Discussion

Zooplankton prey fields

The prey fields available to planktivorous YOY walleye pollock and Pacific herring were different in summer and autumn 1995. Although we report similar total zooplankton densities for the two seasons, we believe that summer densities of small calanoids were underestimated with the 303-μm net. This conclusion is based on a 1995 companion study that showed that a 243-μm mesh net retained small calanoids better than a 303-μm mesh net.[8] The sea-

[8] Sturdevant, M. V. In review.

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sonal densities presented in our study were not directly comparable because of the different gear used, and our summer mesozooplankton densities were lower than those reported from other summer collections in PWS[9] (Cooney et al., 1981; Celewycz and Wertheimer, 1996). The food supply available to juvenile fish was likely more abundant in summer than in autumn, because other studies in the northeastern Pacific showed a steady decline in zooplankton biomass from summer to winter (Peterson and Miller, 1977; Cooney, 1986; Incze et al., 1997; Foy and Paul, 1999). Zooplankton taxonomic compositions also differed seasonally, mainly in the larger percentages of alternate prey available in autumn.

We found the surface-water feeding environment to be richer in numbers of prey than the deeper water in both seasons. Lower zooplankton density with depth has been reported by other authors in PWS,[10] Shelikof Strait (Napp et al., 1996), off the Oregon Coast (Marlowe and Miller, 1975), and at Ocean Station "P" (Petersen and Miller, 1977). Large calanoids were an exception in autumn, however, when their densities and percentages were greater in the deeper tows, typical of subarctic locations (e.g. Marlowe and Miller, 1975; Nakatani, 1988; Napp et al., 1996).

High abundances of *Pseudocalanus* spp. and other small calanoids and high biomass of large calanoids are characteristic of neritic locations in subarctic Pacific waters, such as those of PWS in summer (Springer et al., 1989; Coyle et al., 1990; Celewycz and Wertheimer, 1996; Incze et al., 1997). The time of collection for zooplankton is important, however, because the location of peak abundance in the water column varies with their diel vertical migration. Calanoid copepods are usually most abundant at the surface at night, migrating deeper during the day (e.g. Sekiguchi, 1975). Even though most of our samples were collected during the day, it is not surprising that abundances were greater in shallow tows than in deeper tows because *Pseudocalanus* was dominant. Of the species important in our study, *Pseudocalanus newmani* was typically the shallowest in depth distribution, *Calanus pacificus* was intermediate and *Metridia pacifica*, the deepest (e.g. Bollens and Frost, 1989; Frost and Bollens, 1992; Bollens et al., 1993; Bollens et al., 1992b). However, the vertical distribution patterns of these species were influenced by the presence of predators and other factors (e.g. Bollens and Frost, 1989; Bollens et al., 1992b; Frost and Bollens, 1992; Bollens et al., 1993). The seasonal difference in depth distribution of large calanoids could have been due to seasonal vertical migrations (Sekiguchi, 1975; Mackas et al, 1993), differences in sampling time, or a response to the changing light regime in autumn.

Feeding habits

Summer and autumn diets of YOY allopatric and sympatric walleye pollock and Pacific herring in PWS were similar to those reported from other areas and to those of other pollock and herring caught during the study that did not meet our criteria (Sturdevant, unpubl. data; see "Materials and methods" section). In summer, both species consumed the abundant calanoid taxa as well as less abundant small prey. Calanoid copepods were likewise the predominant summer prey of YOY pollock in Japanese waters (Kamba, 1977; Nakatani, 1988), the region of the Kodiak Island-Alaska Peninsula,[11] the Gulf of Alaska and eastern Bering Sea (Grover, 1990, 1991; Brodeur et al., 1997), PWS (Willette et al., 1997; Foy and Norcross, 1999a), and southeastern Alaska.[12] The small calanoid *Acartia clausi* was particularly important in southeastern Alaska diets from August to October,[12] but by late summer in other areas, euphausiids accounted for more prey biomass and calanoids continued to dominate numerically (Merati and Brodeur, 1996). The autumn prey composition of pollock in our study was also similar to that of YOY pollock in the Gulf of Alaska (Merati and Brodeur, 1996; Brodeur et al., 2000), eastern Kamchatka (Sobolevskii and Senchenko, 1996), and southeastern Alaska.[12] In those studies, increased fish size was correlated with decreased predation on small copepods and increased predation on large copepods, larvaceans, and euphausiids. By winter, large copepods virtually disappeared from diets in some areas (Sobolevskii and Senchenko, 1996); chaetognaths and epibenthic prey such as mysids, shrimps, caprellid amphipods, and cumaceans were incorporated in the diet as vertical distributions of the fish changed and pelagic prey became scarce[12] (Merati and Brodeur, 1996; Sobolevskii and Senchenko, 1996; Brodeur et al., 2000). Seasonal changes in prey have also been correlated with change in YOY pollock distribution and the use of different habitats (Nakatani, 1988).

Like pollock, YOY Pacific herring depended on small calanoid prey in PWS and throughout their range. Additional small prey taxa are commonly reported in Pacific herring and other species diets, including invertebrate eggs, barnacle larvae, cladocerans, oikopleurans, and juvenile amphipods and euphausiids[9] (Wailes, 1936; Sherman and Perkins, 1971; Last, 1989; Arrhenius and Hans-

[8] (*continued*) Summer zooplankton density and composition estimates from 20-m vertical hauls using three net meshes. Alaska Fisheries Research Bull., Auke Bay Laboratory, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, AK 99801.

[9] Sturdevant, M. V., and L. B. Hulbert. 1999. Diet overlap, prey selection, and potential food competition among allopatric and sympatric forage fish species in Prince William Sound, 1996. *In* Forage fish diet overlap, 1994–1996, p. 72–100. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 97163C), Auke Bay Laboratory, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, Alaska 99801.

[10] Foy, R. J., and B. L. Norcross. In prep. Nearshore zooplankton community ecology in Prince William Sound, Alaska. J. Plankton Res., Institute of Marine Science, Univ. Alaska Fairbanks, P. O. Box 757220, Fairbanks, AK 99775-7220.

[11] Livingston, P. A. 1985. Summer food habits of young-of-the-year walleye pollock, *Theragra chalcogramma*, in the Kodiak area of the Gulf of Alaska during 1985. Unpubl. manuscr., Alaska Fish. Sci. Center, National Marine Fisheries Center, 7600 Sand Point Way NE, Seattle, WA 98115, 8 p.

[12] Krieger, K. J. 1985. Food habits and distribution of first-year walleye pollock, *Theragra chalcogramma* (Pallas), in Auke Bay, Southeastern Alaska. Unpubl. MS thesis, Univ. Alaska Juneau, 11305 Glacier Hwy., Juneau, AK 99801, 57 p.

son, 1994; Arrhenius, 1996; Haegele, 1997; Willette et al., 1997). In addition to pelagic prey, epibenthic prey such as harpacticoid copepods and gammarid amphipods were important in estuarine habitats of the Fraser River for Pacific herring and coastal Maine for Atlantic herring (Sherman and Perkins, 1971; Blaxter and Hunter, 1982; Levings, 1983, in Lassuy, 1989). Spatial differences were found for diets of Pacific herring from four widespread bays in PWS (Foy and Norcross, 1999a). We found seasonal differences in prey similar to reports by others: euphausiids replaced calanoids in the diets of older juvenile herring compared with younger juvenile herring (Wailes,1936; Lassuy, 1989; Haegele, 1997), and larvaceans (Foy and Norcross, 1999a, 1999b), mysids, and other malacostracans (Foy and Norcross, 1999a; Foy and Paul, 1999) were also more common in autumn. In Auke Bay, Alaska, juvenile herring diets varied with abundance of zooplankton prey taxa in spring and early summer and included large calanoids and euphausiids when they were present (Coyle and Paul, 1992). The marine distribution of the alewife, *Alosa pseudoharengus*, another herring, was correlated with the seasonal distribution, availability, and abundance of its euphausiid prey (Stone and Jessop, 1994).

Prey selection, feeding time, and depth

Segregation by depth is one way to reduce interspecific competition among fish with overlapping distributions (e.g. Jessop, 1990; Arrhenius, 1996). Both pollock and herring perform diel vertical migrations (Smith, 1981; Blaxter and Hunter, 1982), the pattern of which can vary seasonally and ontogenetically (e.g., Kamba, 1977; Lassuy, 1989; Olla et al., 1996; Stokesbury et al., 2000). We observed some interspecific differences in prey selection that may relate to diel vertical distributions of predator and prey, prey preferences, ontogenetic changes in prey size, or different feeding rhythms for both species. Neither species selected small calanoids in summer, and pollock selected taxa that herring did not (large calanoids, pteropods, and larvaceans). The summer herring were located nearshore and at the surface where densities of their main prey were twice as high as deep in the water column and where light for feeding was most intense. In contrast, summer pollock were located in relatively deep water with less light and lower prey densities. Even though small calanoids were the predominant prey of pollock and the predominant zooplankter, they were avoided in relation to their availability. In autumn, both species avoided small calanoids even more strongly than in summer. In autumn, sympatric pollock in shallow water selected large calanoids, mainly *Metrida pacifica*, that were less available than in deeper water, but only the deeper allopatric herring selected them. Young Pacific herring in another study selected *Calanus pacificus* over *Metridia lucens*, perhaps in relation to fine-scale differences in prey depth distributions (Fortier and Leggett, 1983 in Munk et al., 1989; Bollens et al., 1993). Both sympatric herring and allopatric herring strongly selected the larvaceans that were more evenly distributed in the water column. We primarily sampled fish schools that were located acoustically and assumed that the fish would be located where food was available (e.g. Arrhenius and Hansson, 1999). Comparisons of prey selection between seasons are valid because the calculations are based on prey percentages which did not differ between the 243- and 303-µm mesh sizes used.[8]

The less-digested condition of herring stomach contents by day compared with night suggests that the summer fish and autumn allopatric fish were actively feeding and that the autumn sympatric fish were not. The condition of pollock prey and the fact that the fish were not located where their selected prey were in either season suggest that pollock were not actively feeding at the times we sampled in either season. The change in digested state of their prey with time of day also suggests that YOY pollock may seasonally or ontogenetically switch from feeding principally during the day to feeding at night[12] (Merati and Brodeur, 1996; Brodeur et al., 2000).

Past diel studies have reported different patterns of feeding for pollock and various herring species. For example, peak time of feeding for pollock was at midnight or just before dawn in some studies (Brodeur and Wilson,1996a; Merati and Brodeur, 1996; Willette et al., 1997), but another study showed a change in feeding chronology from sunset in small fish to night in larger fish (Brodeur et al., 2000). Peak time of feeding for Atlantic, Baltic, and Pacific herring occurred in the afternoon or evening, with the lowest feeding rates in early morning (e.g. de Silva, 1973; Blaxter and Hunter, 1982; Mehner and Heerkloss, 1994; Willette et al., 1997; Arrhenius, 1998). Changes in prey composition with time of day and ontogeny have also been noted in some studies (e.g. Nakatani, 1988; Grover, 1991; Munk, 1992; Stone and Jessop, 1994) and not others (Brodeur et al., 2000). If co-occurring fish feed at different times, their diets could be highly similar without direct competition because predation on the same prey resources would be temporally or spatially separated. This could be the case with small calanoid prey, which both species fed on in summer, but only herring were co-located at the depth of prey concentration. Different feeding periodicities could result in indirect competition if prey resources are limited, however.

Sampling time could also affect the appearance in the diet of vertically migrating macrozooplankton, such as juvenile-adult euphausiids and hyperiid amphipods, because the vertical locations of peak abundance of predator and prey may not overlap continually. For example, euphausiids could be consumed at night near the surface or during the day near the bottom[12] (Pearcy et al., 1979; Nakatani, 1988). Juvenile euphausiids were rare summer prey in our study and were present only in those trawls taken below the 60-m mean depth of pollock catches.[2] In autumn, the euphausiid and fish distributions were more likely to overlap during the night sampling time (e.g. Bailey, 1989; Bollens et al., 1992a), and euphausiids were indeed a principal prey in terms of biomass, particularly for pollock. Large calanoids and euphausiids could have been consumed at different feedings, particularly if their vertical distributions overlapped with those of the fish at different times. Predation by pollock and herring on euphausiids in areas where these macrozooplankters were not

collected suggests that the fish fed in a different area or at a different time.

Comparison of allopatric and sympatric aggregations

If competition occurs between sympatric species, one would expect that, given similar prey fields, the quantity or quality of prey consumed would improve when fish are allopatric. If such changes are prolonged, growth or survival differences are probable. We found intraspecific changes in diet composition and food quantity for both species from allopatric to sympatric aggregations, and weak interspecific differences in feeding between sympatric fish. Both species of fish avoided small calanoids whether a competitor was present or not, but the proportional use of energetically advantageous taxa (large calanoid, euphausiid, and hyperiid prey) and the selection for large calanoids were lower in the presence of competitors. Comparisons of sympatric fish showed that pollock ate proportionally more high energy-producing prey (euphausiid biomass, hyperiid frequency, large calanoid numbers) and selected *Metridia* spp. more strongly than herring. Overall, however, high diet overlap between allopatric and sympatric fish of either species in autumn indicated little change in diet composition owing to sympatry. Because both species of sympatric fish ate less total prey than allopatric fish, quantity, rather than quality of prey decreased in the presence of competitors or perhaps as a density-dependent response. Drastic diet shifts were demonstrated in one study involving fish removal; planktivorous species became benthivorous when their competitors were removed from a lake (Persson and Hansson, 1999). In our study, however, although the intraspecific comparisons (allopatric-sympatric) were within month, they differed by depth, time, or region; the interspecific comparisons of allopatric fish were a month apart in different years. Many authors have found differences in zooplankton prey fields on these scales[10] (e.g. Springer et al., 1989; Celewycz and Wertheimer, 1996), making it difficult in our study to separate the effects of sampling differences from those due to competition on diet and feeding.

Seasonal feeding

We observed the highest frequency of empty stomachs for both species in the November 1994 samples; empty stomachs, however, were more frequent for herring than for pollock. Similarly, in other seasonal studies, the proportion of empty stomachs in YOY herring peaked in winter (Foy and Norcross, 1999a; Foy and Paul, 1999), but empty stomachs were never observed among YOY pollock, even though stomachs were least full in December.[12] As zooplankton biomass declined between the winter months of October and February, the feeding response and whole body energy content of herring also declined (Foy and Paul, 1999). The herring relied on stored energy to overwinter (Paul et al., 1998a; Paul and Paul, 1998), but pollock are thought to allocate energy from year-round feeding for somatic growth (Paul et al., 1998b).

We also observed declines in zooplankton coincident with feeding declines in autumn; conversely, a greater food supply of small calanoids supported trends toward more intensive feeding in summer. Feeding on small calanoids in summer could have been density dependent because this taxon was found to be most prominent in the diets when it was more abundant in zooplankton samples. Juvenile Baltic herring exhibited a similar functional response to greater food densities (Arrhenius and Hansson, 1999). However, decreased feeding on small calanoids is more likely related to fish size and energy requirements. Both species (especially pollock in November 1994) were larger in autumn when they ceased consuming small calanoids, and both species avoided them more often. Larger prey are more efficient sources of energy for larger fish, if available. The larger autumn fish were probably better able to prey on late stages of macrozooplankton than the summer fish (Merati and Brodeur, 1996; Haegele, 1997; Kamba, 1977), in synchronism with a seasonal difference in their abundance and availability[13] (Bollens et al., 1992a; Stone and Jessop, 1994; Incze et al., 1997). A shift to larger prey is consistent with a decrease in prey numbers without a change in prey weight because fewer large prey are needed for equivalent weight. The biomass percentages of large prey consumed (large calanoids and euphausiids) and the numeric percentages of small prey consumed (larvaceans) both increased in autumn compared with summer (see also Foy and Norcross, 1999a). Ontogenetic changes and size-selective feeding have been reported by others, as well, for juvenile herring (e.g. Raid, 1985; Munk, 1992; Arrhenius, 1996) and juvenile pollock (Grover, 1991; Brodeur, 1998).

In conjunction with the seasonal diet transition, total prey number and most other measures of quantity were lower in autumn than in summer, particularly for herring. Autumn declines in juvenile pollock feeding rates were also observed in a study in Southeast Alaska in conjunction with a switch from small prey to larger prey.[12] Similarly, feeding declined seasonally for Atlantic herring (de Silva, 1973), Baltic herring (Arrhenius and Hansson, 1999), and Pacific herring (Foy and Norcross, 1999a). In our study, pollock consumed well above maintenance ration (0.30 %BW at 7.5°C; Smith et al., 1986) in all time periods, but, the low prey %BW of herring in autumn 1994 could indicate starvation, given a ration of 1.3–3.6 %BW at 6.2–8.7°C (de Silva and Balbontin, 1974; Arrhenius and Hansson, 1994). Continued feeding and better fish condition late in the year are advantageous for survival through the extreme conditions of winter, and relate to the species different strategies for overwintering.

Declines in feeding with season were also indicated by increased diet overlap between sympatric species in November compared with overlap in October, which suggests a density-dependent convergence of feeding with declining prey resources in late autumn. For sympatric fish in au-

[13] Mooney, J. R. 1999. Distribution, energetics, and parasites of euphausiids in Prince William Sound, Alaska. Unpubl. MS thesis, Juneau Center School of Fisheries and Ocean Science, Univ. Alaska Fairbanks, 11122 Glacier Hwy., Juneau, AK 99801, 172 p.

tumn, lower diet overlap occurred when pollock were more selective for large calanoids and ate proportionally more euphausiids, and the herring selected larvaceans (October 1995); greater dietary overlap in autumn occurred when the species ate similar percentages of these taxa and small calanoids (November 1994).

Despite larger size in autumn, energy requirements of fish are lower during this period of less abundant food due to environmental changes. In PWS, surface temperatures declined from approximately 12°C in summer, to 10°C in October 1995, and 7–8°C in November 1994.[2] Temperatures below the thermocline were stable at 5–6°C, but thermocline depth varied seasonally, between approximately 40–60 m in summer and 50–90 m in either autumn.[3,4] Additional seasonal changes in the environment have been reported by Stokesbury et al. (1999). From summer to winter in PWS, surface waters inside bays changed from being colder to being warmer than surface waters outside bays. Such environmental temperature changes in both the vertical and horizontal planes may influence the distribution of pollock and herring, their energy budgets (Smith and Paul, 1986), and the seasonal distribution and abundance of their prey.

Fish can conserve energy during times of reduced prey by altering behaviors to maximize prey searching or to decrease metabolic costs. For example, fish can alter school cohesiveness (Brodeur and Wilson, 1996b; Stokesbury et al., 1999), restrict their movement, or shift in residence to deeper water with colder temperatures (Sogard and Olla, 1996; Ciannelli et al., 1998). However, they may do so at the risk of increased predation or decreased light for feeding. Herring are primarily visual feeders, requiring minimum light levels to feed (Blaxter and Hunter, 1982) but may also filter feed in low light (Batty et al., 1986; Stone and Jessop, 1994). They can respond to prey distributions correlated with thermocline depth (Fossum and Johannessen, 1979) and may shift feeding during the day to depths where light is not restrictive even if prey are concentrated elsewhere (Munk et al., 1989). Similarly, the vertical distribution of juvenile pollock is affected by the relative availability of food and by numerous other factors that affect feeding conditions: predator presence, light, turbidity, pressure, temperature, size, and metabolic requirements (Bailey, 1989; Olla et al., 1996; Sogard and Olla, 1996; Ciannelli et al., 1998). Juvenile pollock avoided light more and avoided cold water less with growth, especially under conditions of low zooplankton (Olla et al., 1996). Both juvenile pollock (Smith et al., 1986) and YOY herring (de Silva and Balbontin, 1974; Arrhenius and Hansson, 1994) decreased prey consumption at colder temperatures; pollock also had lower maintenance rations and grew more rapidly under conditions of low food with colder temperatures (Smith et al., 1986). Competition with pollock for food could compromise the winter survival of YOY herring by limiting the accumulation of their energy stores.

Research showing major differences in the nutritional quality of forage species consumed by piscivores has sparked interest in their trophic interactions. Pollock lipid content was low compared with that of herring, but unlike herring, was not correlated with size (Anthony and Roby, 1997; Payne et al., 1999; Anthony et al., 2000). However, only a few studies have examined the energetic consequences of feeding on different prey for juvenile pollock (e.g. Smith et al., 1986; Davis and Olla, 1992; Ciannelli et al., 1998) and herring (e.g. de Silva and Balbontin, 1974; Arrhenius and Hansson, 1999). Because prey differ in nutritional composition, caloric density (Ikeda, 1972; Lee, 1974; Deibel et al., 1992; Davis et al., 1998), size, mobility, and behavior, their relative abundance is not the only factor of importance. For example, larvaceans are a highly visible taxon (Bailey et al., 1975) with caloric value per unit weight similar to that of crustacean zooplankton even though they are gelatinous (Davis et al., 1998), but many more larvaceans must be consumed to accumulate the equivalent calories obtained from the crustaceans. Davis and Olla (1992) showed in a controlled experiment that larval pollock growth, behavior, and lipid concentration were affected by the nutritional quality of prey. In a field study, herring diets had the highest energy density of all in May, when large calanoids were the most important taxon (Foy and Norcross, 1999a); however, they were not examined from late fall—a period for which our study showed that euphausiids were the prominent prey and others have shown they contain higher energy density compared with earlier times of the year.[13] If sympatry induces feeding on prey of lesser nutritional quality for extended periods because of interference competition, fish growth and survival could be affected.

For sympatry to occur, the distribution of juvenile walleye pollock and Pacific herring must overlap in three dimensions: time (seasonal and diel), and both horizontal and vertical space. These species have different life histories (Smith, 1981; Lassuy, 1989) and patterns of movement change ontogenetically, suggesting that spatial overlap is likely to vary. YOY herring generally school near the bottom along shore during the day, then move up to the surface at dusk and disperse (Blaxter and Hunter, 1982; Lassuy, 1989; Haegele, 1997). Early YOY pollock stay principally in surface water above the thermocline, perform a diel vertical migration (DVM), and disperse or move inshore at night; depth distribution increases from summer to autumn[12] and with ontogeny (e.g. Nakatani, 1988; Karp and Walters, 1994; Olla et al., 1996). Stokesbury et al. (1999) found that herring and pollock were generally depth stratified but that both were aggregated in bays in July and October. Rather than having a strong species association, they may simply have an affinity for the same habitats at some points in their life histories (Brodeur and Wilson, 1996a). Data collected monthly in PWS in 1994 (Willette et al., unpubl. data) showed that, after May, >50% of juvenile herring sets also caught juvenile pollock, and after July, >50% of juvenile pollock sets also caught juvenile herring. Willette et al. (1997) noted that diet overlap was more than twice as great for pollock and herring from sympatric sites than for these species from allopatric sites in late summer. These patterns suggest that sympatry and feeding competition increase from spring through summer.

Our study is the first to examine the feeding interactions of juvenile walleye pollock and Pacific herring. Its limited scope, because it was designed for other primary

purposes, necessitates a primarily descriptive approach. Differences that we observed in fish feeding could relate to variable sampling intensity, limited fish sample sizes, gear limitations for certain prey taxa, spatial variation in prey, or differences in fish depths. Nonetheless, the similarity of dietary requirements between YOY pollock and herring could induce competition for limited food when these fish co-occur during periods or in places of low food availability, particularly late autumn and winter. Stokesbury et al. (1999) showed spatial variation in growth of YOY herring among widespread bays in PWS and surmised that density-dependent interspecific competition for food was one variable that affected growth rates; likewise, Paul and Paul (1999) showed interannual and spatial variations in size and energy content of YOY pollock from PWS and speculated that prey production and delivery contributed to the differences. The densities of planktivorous predators may exceed the carrying capacity of bays where walleye pollock and Pacific herring are sympatric, adding interspecific competition to the factors limiting growth. More studies comparing spatial and temporal patterns of distribution, abundance, and feeding are needed to clarify the extent and frequency of interactions between YOY pollock, herring, and other forage species and the impact of changes in prey and climate on these interactions.

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Abstract—Bigeye tuna (*Thunnus obesus*) age and growth studies were last conducted in the western Pacific in 1967 and no study has ever attempted to age bigeye tuna from this area by using dorsal spines. The objective of our study was to estimate bigeye tuna age and growth rate in the western Pacific based on counts of growth rings on sections of the first dorsal spine. Length and weight data, and the first dorsal spine from bigeye tuna in the Tungkang (southwest of Taiwan) fish market were collected monthly from February 1997 to January 1998. In total, 1149 specimens were collected. The fork lengths of individuals ranged from 45.6 to 189.2 cm. Cross sections from dorsal spines were taken and examined under a dissecting microscope equipped with an image analysis system. The monthly percentage of specimens having a terminal translucent zone indicated that growth rings formed once a year; therefore, the age of each fish was determined from the number of visible growth rings. Von Bertalanffy growth parameters were estimated for males, females, and both sexes combined. There was no significant difference between males and females. The parameter estimates for the combined sexes were asymptotic length (L_∞) = 208.7 cm, growth coefficient (K) = 0.201/yr, and age at zero length (t_0) = –0.9906 yr.

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Age and growth of the bigeye tuna, *Thunnus obesus,* in the western Pacific Ocean

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Bigeye tuna (*Thunnus obesus* Lowe, 1839) are a commercially important species of tuna inhabiting the warm waters of the Atlantic, Indian, and Pacific oceans. They are found across the entire Pacific between northern Japan and North Island of New Zealand in the west and from 40°N to 30°S in the east (Calkins, 1980; Matsumoto, 1998). Adult bigeye tuna are caught mainly by longlines, but substantial numbers of juveniles are taken by purse seines.

Taiwanese distant water tuna longline fleets have operated throughout these three oceans since the late 1960s targeting albacore. In the early 1980s, the Taiwanese began equipping their longliners with very cold (below –55°C) freezers and deep longlines in the Indian and Atlantic oceans, which allowed them to target bigeye tuna for the lucrative sashimi market in Japan. In the western Pacific, the Taiwanese offshore longline fleets, based in domestic (Tungkang mainly) and foreign fishing ports, have landed more bigeye tuna than in the past.

Growth studies of Pacific bigeye tuna conducted in the 1950s and 1960s were based either on increments between modal points in size-composition data (Iversen, 1955; Shomura and Keala, 1963; Yukinawa and Yabuta, 1963; Kume and Joseph, 1966; Suda and Kume, 1967) or on the number of annual markings (annuli) on scales (Nose et al., 1957; Yukinawa and Yabuta, 1963). Recently, Hampton and Leroy[1] and Matsumoto (1998) presented preliminary results from growth studies based on otolith increment counts. No previous study had aged Pacific and Indian bigeye tuna from dorsal spines, although a few age determination studies existed for Atlantic bigeye tuna (Gaikov et al., 1980; Draganik and Pelczarski, 1984; Delgado de Molina and Santana, 1986; Alves et al., 1998). Accurate age structure of stocks is essential for stock assessment and fishery management. Our study provides estimates of the age and growth rate of bigeye tuna in the western Pacific from growth rings on sections of the first dorsal spine.

Materials and methods

Fork length (in cm), weight (in kg), and sex were determined for bigeye tuna caught by Taiwanese offshore longliners in the fishing area from 23°N to 0°N and 110°E to 140°E (Fig. 1[2]) and sold at the Tungkang fish market between February 1997 and January 1998. In addition, a total of 1149 first dorsal spines were collected. Three cross sections were taken along the length of each spine above the condyle base (Fig. 2A) with a low-speed "ISOMET" saw

[1] Hampton, J., and B. Leroy. 1998. Note on preliminary estimates of bigeye growth from presumed daily increments on otoliths and tagging data. Working paper 18, eleventh meeting of the standing committee on tuna and billfish, Honolulu, Hawaii, USA, 30 May–6 June 1998, 3 p. Oceanic Fisheries Programme, Secretariate of the Pacific Community, B.P.D5, 98848 Noumea, New Caledonia.

[2] Yang, R. T., R. F. Chung, and C. L. Chang. 1982. Taiwanese offshore tuna longline fishery. Part I: fishing ground, fishing season, and fishing condition. Spec. Rep. 36, 6 p. [In Chinese with English abstract.] Insitute of Oceanography, National Taiwan University, no. 1, sec 4, Roosevelt Rd, Taipei, 106 Taiwan.

(model no. 11-1280) and diamond wafering blades. Sections ranging from 0.8 to 1.0 mm thick (Fig. 2B) were examined with a dissecting microscope (model: Olympus SZH-ILLD) with transmitted light. Images of the dorsal spine sections were captured by using an image analysis software package, a CCD (charged coupled device) camera, and a high-resolution computer monitor. Translucent rings on the section images were counted by two readers independently. When ring counts disagreed, images were read again by both readers simultaneously, and any questionable spines were discarded.

Spine sections as the structure to estimate age have the advantage of requiring easy sampling and easy reading (the growth rings stand out clearly), and samples are easily stored for future reexamination (Compeán-Jimenez and Bard, 1983). However, early growth rings may be lost in larger specimens because of increased size of the vascularized core in the spine. Accordingly, we estimated the number of lost (obscured) rings from observations of their position and number in spines from young specimens as has been done for little tunny (*Euthynnus alletteratus*) (Cayré and Diouf, 1983), eastern Atlantic bluefin tuna (*Thunnus thynnus*) (Compeán-Jimenez and Bard, 1983), and Pacific blue marlin (*Makaira nigricans*) (Hill et al., 1989).

Age was determined from the translucent rings, assuming that two rings are formed each year—a translucent (light colored) ring formed during the slower growth period and an opaque (dark colored) ring formed during the fast growth period. This assumption was validated by observing a translucent or opaque edge on the dorsal-spine sections and a monthly variation in the number of translucent edges (Antoine et al., 1983).

Distance between the center of the dorsal spine and the outer edge of each annual ring was measured in microns with the software package after calibration against an optical micrometer. The center of the spine was estimated by following Cayré and Diouf (1983) (Fig. 2B). Distances (d_i) were then converted into radii (R_i) by following González-Garcés and Fariña-Perez (1983).

The relationship between fork length (FL) and dorsal spine radius (R) was modeled by a linear equation (Zar, 1999). Fork length was then back-calculated for each ring with the formula (Lee, 1920)

$$FL_i = a + \frac{(FL - a)R_i}{R},$$

where FL_i = predicted fork length of the fish corresponding to age or ring i in cm;
a = ordinate in the origin of the equation $FL = a + bR$;

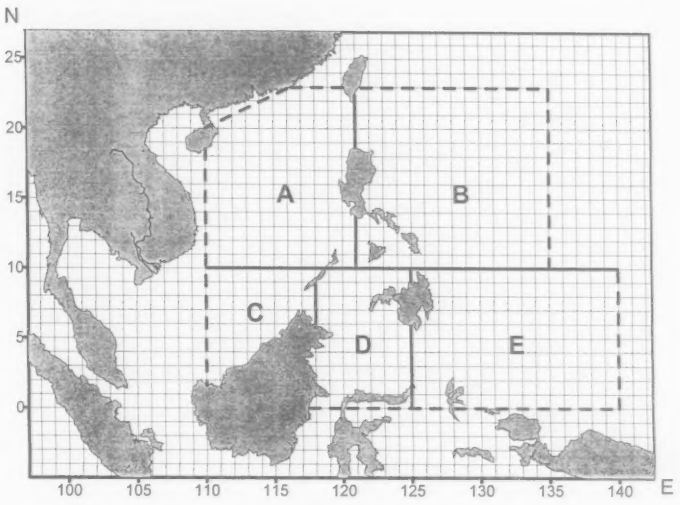

Figure 1

Fishing areas of the Taiwanese offshore tuna longline fishery in the western Pacific Ocean (Yang et al.[2]).

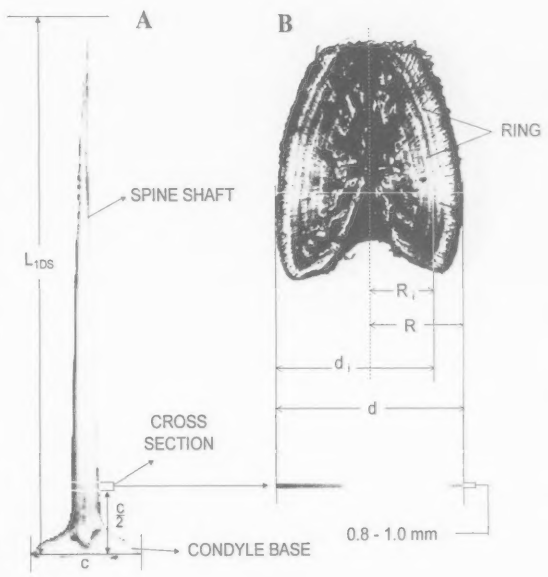

Figure 2

First dorsal spine and the site of cross section (**A**) and the cross section showing annual rings and measurements taken (**B**) for age determination of the western Pacific bigeye tuna (c =width of condyle base; L_{1DS}=length of the first dorsal spine; R=radius of spine; R_i=radius of ring i; d=diameter of spine; d_i=diameter of ring i).

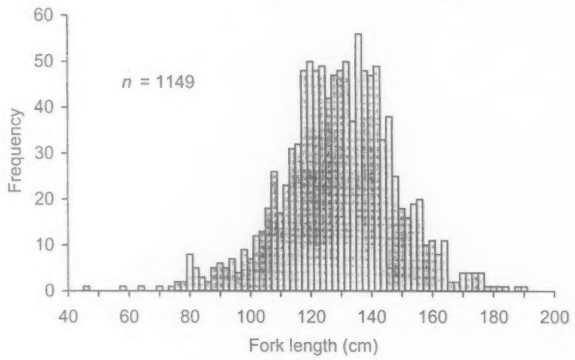

Figure 3

Length-frequency distribution for the western Pacific bigeye tuna sampled at Tungkang fish market from February 1997 to January 1998.

FL = observed fork length of the fish in cm;
R_i = radius of the ring calculated as the average value observed in ring i (Fig. 2B); and
R = dorsal spine radius.

Back-calculated fork lengths were used in Ford-Walford (Gulland, 1983) and nonlinear (Ratkowsky, 1983) methods to fit the von Bertalanffy growth function (VBGF) and to obtain vital parameters by sex. Analysis of the residual sum of squares (ARSS) was employed to compare the VBGF between sexes (Ratkowsky, 1983; Chen et al., 1992).

Weight was related to fork length by using the power function, and analysis of covariance (ANCOVA) (Steel, 1980; Zar, 1999) was conducted to examine differences between sexes.

Results

Spines from 1149 specimens ranging in size from 45.6 to 189.2 cm FL were examined (Table 1, Fig. 3). There was 90% agreement between the readers' counts of growth rings and second readings improved this agreement to 95.6%, which resulted in discarding 51 specimens from analysis.

The relationship between FL (cm) and weight (kg) is shown in Figure 4. The ANCOVA indicated no significant difference between males and females ($P>0.05$); thus the FL-W relationship with sexes combined was expressed as

$$W = 3 \times 10^{-5} FL^{2.9278} \qquad (r^2=0.97, n=856).$$

The relationship of first dorsal spine lengths (L_{1DS}) and FL was (Fig. 5)

Table 1

Sample sizes, ranges of fork lengths (FL, cm), and sampling months and areas of bigeye tuna from the western Pacific Ocean. A, B, C, D, and E denote areas in Figure 1.

Month	Sampling area	Sample size	Minimum FL	Maximum FL
Feb 1997	A	80	70	174.5
Mar 1997	A	104	64	169.5
Apr 1997	B	70	101.3	171
May 1997	B	54	83.8	157.4
Jun 1997	B	71	75.5	162.8
Jul 1997	B, D	131	72.2	165.6
Aug 1997	E	94	78.5	187.7
Sep 1997	E	98	45.6	189.2
Oct 1997	A, C	115	86.5	176.6
Nov 1997	A, C	116	89.6	161.1
Dec 1997	C	123	104.6	162.1
Jan 1998	A	93	88.7	159.1
Total		1149	45.6	189.2

$$FL = 6.9367 L_{1DS} + 6.6667 \qquad (r=0.94, n=567).$$

The trend of the monthly percentages of terminal translucent edges (Fig. 6) suggested that the period from February to September was the long period of inhibited growth (translucent edge). From October to November, growth appeared to resume (opaque edge) and later, from December to January, a new translucent edge appeared; indicating the formation of one growth ring per year.

Given the significant linear relationship between the dorsal spine radius and fork length (FL=26.455R + 19.916, r=0.94, n=1098), we used spine measurements to back-

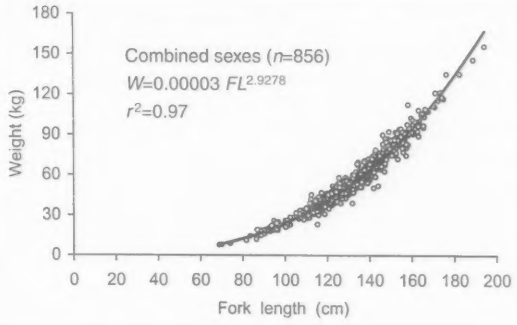

Figure 4

Relationships between weight and fork length of the western Pacific bigeye tuna sampled at Tungkang fish market.

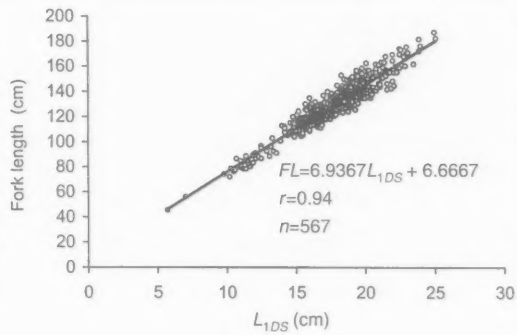

Figure 5

Relationship between fork length and length of the first dorsal spine (L_{1DS}) of the western Pacific bigeye tuna sampled at Tungkang fish market.

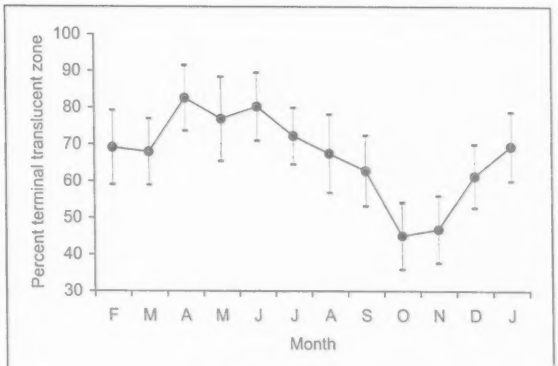

Figure 6

Monthly variation in percentage of the western Pacific bigeye tuna with a terminal translucent zone in dorsal spine sections, February 1997 to January 1998.

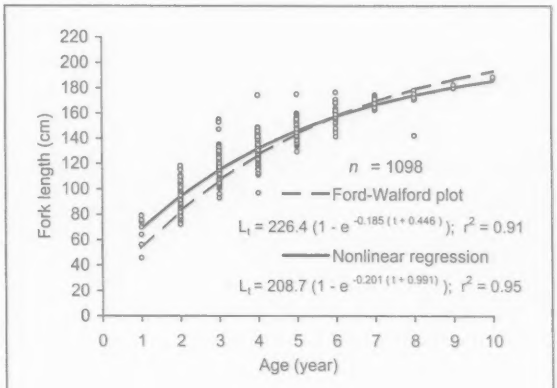
n = 1098
Ford-Walford plot
Lt = 226.4 (1 - e -0.185 (t + 0.446)); r2 = 0.91
Nonlinear regression
Lt = 208.7 (1 - e -0.201 (t + 0.991)); r2 = 0.95
Fork length (cm)
Age (year)

Figure 7

Comparison of the growth curve obtained by the Ford-Walford plot with the growth curve obtained by nonlinear regression method for the western Pacific bigeye tuna.

calculate the fork lengths of previous ages. The mean back-calculated fork lengths for the first 10 years of life for the western Pacific bigeye tuna are given in Table 2.

Parameters of the VBGF estimated by the Ford-Walford method for males, females, and sexes combined are shown in Table 3. Growth was not significantly different between sexes (ARSS, F=1.98; df=3, 452; P>0.05); the pooled growth curve is shown in Figure 7. VBGF parameters computed by nonlinear regression are also shown in Table 3 and Figure 7. Length-at-age of bigeye tuna estimated by nonlinear regression is larger (up to age 6 years) than that estimated by the Ford-Walford method.

Discussion

Available genetic information supports the hypothesis of a single bigeye stock in the Pacific Ocean (Hampton et al., 1998; Grewe and Hampton[3]). Although the fishing area of the Taiwan fleet and thus the sampling area of bigeye tuna used in our study was limited to a small area of the western Pacific, our results may be representative of bigeye tuna throughout the Pacific Ocean.

Monthly variation in percent terminal translucent edges in our study suggested the formation of growth rings once a year. Ehrhardt et al. (1996) attributed the narrow, trans-

[3] Grewe, P. M., and J. Hampton. 1998. An assessment of bigeye (*Thunnus obesus*) population structure in the Pacific Ocean, based on mitochondrial DNA and DNA microsatellite analysis. University of Hawaii, Joint Institute for Marine and Atmosphere Research Contribution 98-320, 29 p. Pelagic Fisheries Research Program, University of Hawaii at Manoa, 1000 Pope Road, Honolulu, HI 96822.

Table 2

Observed and back-calculated mean fork length (FL, cm) at age for the western Pacific bigeye tuna, *Thunnus obesus*. ("—" means there were no data owing to vascularization at core area). Numbers in normal print represent the mean back-calculated fork lengths; numbers in parentheses represent the number of specimens for which the specified ring was readable.

Age (yr)	*n*	Observed mean FL (cm)	Annulus number									
			I	II	III	IV	V	VI	VII	VIII	IX	X
1	8	67.9	57.0 (8)									
2	79	93.7	50.0 (29)	80.8 (79)								
3	329	115.3	52.4 (69)	85.8 (265)	102.9 (329)							
4	413	131.7	51.9	82.7 (19)	107.1 (162)	121.2 (384)	(413)					
5	188	145.9	—	80.9 (14)	112.1 (77)	122.8 (184)	135.8 (188)					
6	59	158.4	—	—	115.5	126.6 (3)	138.2 (36)	149.5 (58)	(59)			
7	11	169.3	—		119.5 (1)	130.1 (5)	142.6 (9)	152.1 (11)	161.8 (11)			
8	6	174.7	—	—	—	123.8 (1)	139.6 (5)	150.8 (6)	161.0 (6)	172.1 (6)		
9	3	178.5	—	—	—	121.0 (1)	129.2 (2)	142.0 (2)	154.2 (3)	169.2 (3)	174.2 (3)	
10	2	188.5	—	—	—	—	138.2 (1)	148.4 (2)	162.5 (2)	174.1 (2)	180.6 (2)	186.2 (2)
Total	1098		(125)	(520)	(794)	(640)	(263)	(80)	(22)	(11)	(5)	(2)
Weighted back-calculated mean FL (cm)			52.1	83.9	105.9	122.0	136.6	149.8	160.6	171.7	177.4	186.2
Growth increment (cm)			—	31.9	21.9	16.2	14.6	13.1	10.9	11.1	5.7	8.7

Table 3

Growth parameters obtained by the Ford-Walford plot method and the nonlinear regression method for the bigeye tuna from the western Pacific Ocean.

Parameter	Ford-Walford plot				Nonlinear regression
	Male	Female	Pooled	Total[1]	Total[1]
n	278	180	458	1098	1098
K	0.1789	0.191	0.1842	0.185	0.2011
L_∞	220.6	211.4	216.1	226.4	208.7
t_0	–0.5566	–0.4592	–0.5266	–0.4465	–0.9906

[1] Male, female, and sex-unknown combined.

lucent rings to slower growth periods, whereas the wide, opaque rings were attributed to periods of fast growth. The spawning season of bigeye tuna in the western Pacific is between February and September and peaks from March to June (Sun, et al.[4]), the period that we found to coincide

[4] Sun, C. L., S. L. Chu, and S. Z. Yeh. 1999. Note on reproduction biology of bigeye tuna in the western Pacific. SCTB12/WP/BET-4, 6 p. Twelfth meeting of the standing committee on tuna and billfish; Tahiti, French Polynesia, June 14–23, 1999. Oceanic Fisheries Programme, Secretariate of the Pacific Community, B.P.D5, 98848 Noumea, New Caledonia.

Table 4

Comparisons of estimates of parameters of von Bertalanffy growth function for the Pacific bigeye tuna by various authors. Partly reproduced from Table 10 of Shomura (1966).

Area	Investigator(s)	Method of analysis	Parameters			Size range of fish (cm)	Comments
			L_∞ (cm)	K	t_0		
Pacific-wide, north of 10°S	Nose et al. (1957)	Scales	195.2	0.106	–1.128	Mean observed length for 58–109	Parameters based on mean observed length by age provided by authors
Western North Pacific (north of 2°N and west of 180°)	Yukinawa and Yabuta (1963)	Size frequency	257.5	0.156	–0.107	Modal sizes estimated to be 65–150	Parameters computed from data provided by authors
Pacific (north of 10°S)	Yukinawa and Yabuta (1963)	Scales	215	0.10412	0.0010995	Estimate 51–160	Authors' values; time (t) in half-year units
Pacific (north of 10°S)	Yukinawa and Yabuta (1963)	Scales (same basic data as above)	213.1	0.212	0.017		Parameters computed by graphic method from data provided by authors
Central Pacific (Hawaiian Islands)	Shomura and Keala (1963)	Size frequency (males)	196.7	0.267	–0.929		Authors' values
Central Pacific (Hawaiian Islands)	Shomura and Keala (1963)	Size frequency (females)	183.0	0.316	0.718		Authors' values
Eastern Pacific (east of 130°W and between 10°N and 25°S)	Kume and Joseph (1966)	Size frequency	186.95	0.095	2.11	39–209	Authors' values; time (t) in quarter-year units
Pacific area	Suda and Kume (1967)	Size frequency	214.8	0.2066	0.0249		
Western Pacific	Hampton and Leroy (1998)	Otolith and tagging	165.3	0.3732	0.3420	25–175	Preliminary
Eastern Pacific	Matsumoto (1998)	Otolith	—	—	—	33.4–57.9	Author estimated only the lengths of 40 and 55 cm at ages of 0.5 and one year, respectively; preliminary.
Western Pacific	This study	Spine	208.7	0.2011	–0.9906	45.6–189.2	

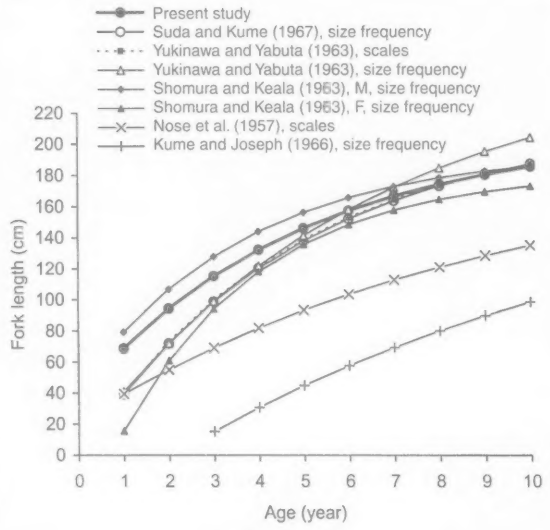
Present study
Suda and Kume (1967), size frequency
Yukinawa and Yabuta (1963), scales
Yukinawa and Yabuta (1963), size frequency
Shomura and Keala (1953), M, size frequency
Shomura and Keala (1953), F, size frequency
Nose et al. (1957), scales
Kume and Joseph (1966), size frequency
Fork length (cm)
Age (year)

Figure 8

Growth curves of Pacific bigeye tuna estimated by different authors.

with the slow-growth period indicated by the narrow and translucent rings. Similar findings have been reported for skipjack tuna (Antoine et al., 1983), bigeye tuna (Gaikov et al., 1980), and swordfish (Ehrhardt, 1992; Tserpes and Tsimenides, 1995). Our efforts only partially validate fish age; complete validation requires either mark-recapture data or the study of known-age fish in the population (Beamish and McFarlane, 1983; Prince et al., 1995; Tserpes and Tsimenides, 1995).

We estimated the parameters of the VBGF by using the Ford-Walford and nonlinear methods and found that the nonlinear method had a better fit (r^2=0.95) than the Ford-Walford method (r^2=0.91). Comparisons of our VBGF parameters with previous studies (Fig. 8, Table 4) showed similar results to those of Yukinawa and Yabuta (1963) who used scales and to those of Suda and Kume (1967) who also used Pacific samples of bigeye tuna. The values of t_0 differed because different aging techniques were used. Following the suggestion of Gallucci and Quinn (1979), Vaughan and Kanciruk (1982), and Hanumara and Hoenig (1987) that Ford-Walford and other linear methods be replaced by nonlinear fitting techniques; we propose using parameters of VBGF estimated by the nonlinear method (Table 3) for description of age and growth for the western Pacific bigeye tuna.

Acknowledgments

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Nocturnal occurrence of the swimming crab *Ovalipes punctatus* in the swash zone of a sandy beach in northeastern Japan

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Swimming crabs belonging to the genus *Ovalipes* (Crustacea: Brachyura: Portunidae) are distributed worldwide along sandy coastlines of subtropical and temperate waters (Stephenson and Rees, 1968). They are extremely well adapted to life on sand, with their strong swimming and burrowing abilities (Brown and McLachlan, 1990). Crabs of this genus are voracious carnivores and significant predators of commercially important mollusks on sandy beaches (Du Preez, 1984; Brown and McLachlan, 1990). Recently, interest in their importance as a possible fisheries resource has increased (Sasaki and Kawasaki, 1980; Wear and Haddon, 1987).

Ovalipes punctatus (De Haan) is common in the coastal waters of Japan and China. It is found on sandy bottoms below the intertidal zone, but peak abundance is at 5–60 m. This species has an ontogenetic migration into deeper water (Kamei, 1976; Sasaki and Kawasaki, 1980). Sasaki and Kawasaki (1980) investigated the life history of *O. punctatus* in Sendai Bay (100 km south of the present study site), on the Pacific coast of northeastern Japan. Female crabs spawn from mid-September until mid-November in offshore water 40–60 m deep. The incubation period of the eggs is about 20 days. After a planktonic phase, the larvae settle on sandy bottoms in March and April. Settled crabs of both sexes grow to carapace widths (CW) of 50–65 mm in the first year. In the second year, females and males grow to 70–90 and 80–85 mm CW, respectively. The life span of this crab is 2 to 2.5 years. The minimum size at maturity is estimated to be 45–50 mm CW (Sasaki and Kawasaki, 1980), but the spawning population consists mainly of 2-year-old crabs of 75–80 mm CW. However, knowledge of the diet and feeding habits of this species has been extremely limited until now.

Ovalipes species, including *O. punctatus*, generally live below the intertidal zone. However, they are often found in the intertidal zone, especially at night (McLachlan et al., 1979; Brown and McLachlan, 1990). McLachlan et al. (1979) suggested that this occurrence is related to feeding, but details are still unknown. During the course of an ecological study of the sandy beach in Otsuchi Bay, we frequently found *O. punctatus* in the swash zone at night. This note examines the significance of the swash zone in the life history of the swimming crab *O. punctatus* in relation to feeding and molting.

Materials and methods

The study was conducted at Koshirahama Beach, in Otsuchi Bay, on the Pacific coast of northern Honshu, mainland Japan, in September of 1994 and August of 1995 (Table 1). The beach is about 120 m long, bounded on both ends by rocky shores, and is categorized as a "sheltered beach" according to McLachlan's (1980) rating scheme. Mean depth at 20, 50, and 100 m from the shoreline is 1.6, 3.5, and 5.5 m, respectively. The beach has a 1:13 (rise: run) slope and median particle diameter of the sediment is 260 µm. Other details of the study site are given in Takahashi and Kawaguchi (1995).

Crabs were sampled with a beach seine 1.1 m high by 8.3 m wide with 1.0-mm mesh. The net was hauled parallel to the shoreline at a depth of ca. 0–1 m. Samples were taken along the entire shoreline of Koshirahama Beach. All crabs were preserved in 10% formalin, and the carapace width (CW), sex, maturity and molt stage of each individual were recorded. The foregut was removed and stored in 70% ethanol for dietary analysis. Sexual maturity of *O. punctatus* was assigned by size classes as defined by Sasaki and Kawasaki (1980): juvenile (<30 mm CW), immature (31–50 mm CW), or adult (>51 mm CW). Molt stages were based on the criteria of Drach and Tchernigovtzeff (1967) and Norman and Jones (1992): 1) soft, no calcification of the new exoskeleton; 2) early papershell, thin, flexible exoskeleton, easily depressed when touched; 3) late papershell, hard exoskeleton except for the branchiostegite region which is compressible; 4) intermolt, completely hard exoskeleton; and 5) premolt, teeth on chelae well worn and having a less rounded appearance compared with those in earlier stages, complete exocuticle developed beneath the exoskeleton.

The diet of *O. punctatus* was analyzed by using the points method and the percentage occurrence method (Williams, 1981; Wear and Haddon, 1987). The points method assesses diet composition in terms of both foregut fullness and estimated volume of food in the foregut. First, the relative degree of foregut fullness of each crab was estimated visually by using six ordered classes (empty=class 0; trace=class 1; 25%=class 2; 50%=class 3; 75%=class 4; full=class 5). A visual assessment of fullness was possible because, except for the gastric mill, the foregut of *O. punctatus* is like a thin-walled translucent bag.

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Fish. Bull. 99:510–515 (2001)

Table 1

Details of the sampling dates and times (JST) and results in the swash zone of Koshirahama Beach, Otsuchi Bay.

Date	Day or night	Sampling time (h)	No. of crabs collected
22 Aug 1995	Day	8:02	3
25 Aug 1995	Day	13:12	2
30 Aug 1995	Day	13:15	3
12 Sept 1994	Night	20:54	30
25 Aug 1995	Night	21:06	23
30 Aug 1995	Night	20:48	35

Food items were identified to the lowest possible taxon under a binocular dissecting microscope. The relative contribution of each prey category to the total volume of the foregut contents was assessed subjectively in the following way: a category representing 95–100% of the total contents was awarded 100 points; 65–95%, 75 points; 35–65%, 50 points; 5–35%, 25 points; 5% or less, 2.5 points; empty, 0 points. The points that each prey category received were weighted by multiplying by a factor that depended on the degree of foregut fullness, i.e. full = 1, 75% = 0.75, 50% = 0.5, 25% = 0.25 and trace = 0.02 (Wear and Haddon, 1987). The maximum and minimum weighted points possible for a single category in a single foregut were 100 (100 × 1.0) and 0.05 (2.5 × 0.02), respectively. The following percentages were calculated for each prey category (Williams, 1981):

$$\text{Percentage points for } i\text{th prey} = \left(\sum_{j=1}^{n} a_{ij} \Big/ A\right) 100; \text{ and}$$

$$\text{Percentage occurrence for } i\text{th prey} = (b_i/N)100,$$

where a_{ij} = the number of points for prey item i in the foregut of the jth crab;
A = the total points for all the crabs and all the prey items in all the foreguts examined;
N = the number of crabs examined with food in the foregut; and
b_i = the number of crabs with foreguts containing prey category i.

Results

Diel change in the swash zone occurrence pattern

A total of 96 crabs (41 males, 55 females), ranging from 12 to 80 mm CW, were collected from the swash zone of Koshirahama Beach (Fig. 1). No ovigerous females were collected.

Almost all specimens were collected at night (88 individuals, 92% of the total catch) and only 8 crabs were collected during the day (Fig. 1). Juvenile (35) and immature

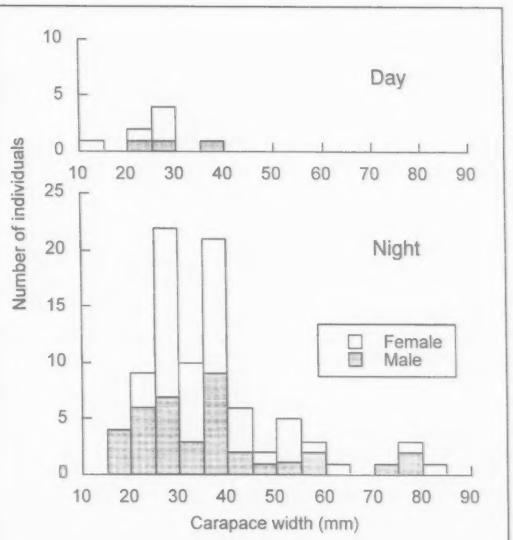

Figure 1

Histograms of carapace width for the swimming crab *Ovalipes punctatus* found in the swash zone of Koshirahama Beach, Otsuchi Bay, during the day and at night. All the specimens were collected in September 1994 or August 1995.

(39) crabs dominated the nocturnal catches, accounting for 40% and 44% of the total nocturnal catch, respectively (Table 2). Fourteen adult crabs were also caught at night, constituting 16% of the nocturnal samples (Table 2). Daytime catches consisted of 1 immature and 7 juvenile crabs (Table 2). On average, crabs caught at night (36 mm CW) were significantly larger than crabs caught during the day (25 mm CW) ($P<0.01$; t-test).

All crabs were in the intermolt or the papershell stages (early and late papershell); no soft-shelled and premolt crabs were collected. During the daytime, 7 out of 8 crabs were in the papershell stages and only one individual was classified as intermolt (Table 2). At night, 5 juvenile and 5 immature papershell crabs were collected, accounting for 14% and 13% of the total catch for each category, respectively (Table 2). Of the adults collected at night, 9 were in the papershell stages, accounting for 64% of the total adult crabs taken.

Feeding activity and diet composition

Because feeding habits of early and late papershell crabs are not different from those of intermolt stages in portunid crabs (Norman and Jones, 1992), analysis for foregut fullness and diet was conducted by pooling all molt stages. The percent frequency of each class of foregut fullness is shown separately for day and night in Figure 2. Although the daytime sample was small, 50% of the crabs had empty foreguts (Fig. 2), but at night over 70% of the

Table 2

Ovalipes punctatus caught in the swash zone of Koshirahama Beach, Otsuchi Bay, during the summers of 1994 and 1995. The numbers of each developmental stage and of papershell crabs caught during the day and at night are shown. No softshell and premolt crabs were collected.

Day or night	Developmental stage of crabs (CW)	Total number of crabs collected	Number of papershell crabs	
			Early papershell	Late papershell
Day	Juvenile (12–30 mm)	7	6	—
	Immature (31–50 mm)	1	1	—
	Adult (51–80 mm)	—	—	—
Night	Juvenile (12–30 mm)	35	4	1
	Immature (31–50 mm)	39	4	1
	Adult (51–80 mm)	14	3	6

Table 3

Percentage occurrence, points, and percentage points for the 12 categories of foregut content in 69 *Ovalipes punctatus* from Koshirahama Beach, Otsuchi Bay. The points express the dietary contribution in terms of foregut fullness and estimated volume of food items in the foregut (see text). Sand was excluded from the calculation of the percentage points.

Food item	Percentage occurrence	Points	Percentage points
Crustaceans			
Haustorioides japonicus	59	1437.5	26.1
Archaeomysis kokuboi	54	1287.5	23.4
Excirolana chiltoni	30	528.7	9.6
Crangon sp.	14	540.0	9.8
Crustacean fragments	42	1115.6	20.3
Other crustaceans	4	62.5	1.1
Mollusks			
Bivalves	6	41.3	0.7
Gastropods	1	25.0	0.5
Fishes			
Unidentified fish	3	5.0	0.1
Other items			
Unidentified organic matter	19	456.3	8.3
Algae	3	3.8	0.1
Sand	94	970.6	—

foreguts examined were more than half full (classes 3–5) and 7% had empty foreguts, which suggests that the crabs fed actively in the swash zone at night (Fig. 2).

When describing the diet of portunid crabs, Williams (1981) recommended including only individuals with foreguts more than 50% full (i.e. classes 3–5). Applying this criterion, we found 69 crabs (39 females, 30 males) for the diet analysis, 66 of which were collected at night. Because there was no significant difference in the diets by sex (chi-square test, $P>0.5$), the male and female data were combined. Forguts contained 11 prey categories plus sand; percent frequency of occurrence, total points, and the relative proportions of the diet components in terms of points are listed in Table 3. Although sand is not considered a part of the diet, it contributed more than 14% to the total points of the foregut contents.

Small crustaceans were frequent and predominant in the foreguts of *O. punctatus* (Table 3). The sand-burrowing amphipod *Haustorioides japonicus* was the most frequent prey, contributing 26.1% of the total points. Second most frequent prey was the sand-burrowing mysid *Archaeomysis kokuboi* (23.3% of the total points), followed by the sand-burrowing isopod *Excirolana chiltoni* (9.6%) and sand shrimps *Crangon* sp. (9.8%). Fragments that probably came from these crustaceans produced 20.3% of the total points (Table 3). The results show 90.2% of the total diet volume of *O. punctatus* consisted of crustacean prey. Mollusks and fish were not important in the diet (1.3% collectively).

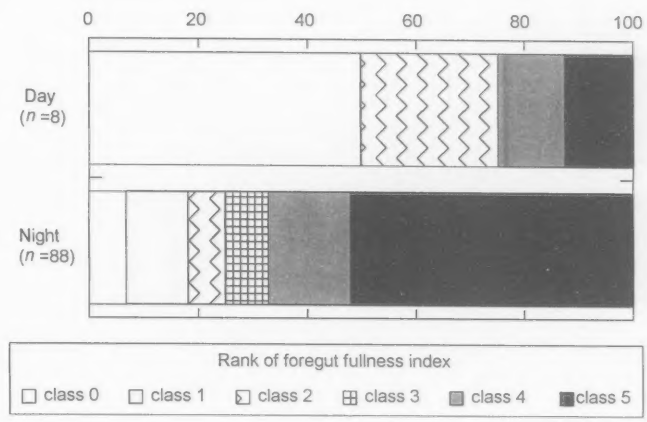

Figure 2

Difference in the foregut fullness index of the swimming crab *Ovalipes punctatus* in the swash zone of Koshirahama Beach, Otsuchi Bay between day and night. Class 0 = empty foregut, class 1 = trace items in foregut, class 2 = foregut 25% full, class 3 = 50% full , class 4 = 75% full, class 5 = full.

Discussion

Ovalipes punctatus occurred nocturnally in the swash zone of Koshirahama Beach, Otsuchi Bay. The nocturnal occurrence in the swash zone for congeners such as *O. trimaculatus* from South Africa (reported as *O. punctatus*, see Schoeman and Cockcroft, 1993) is often observed elsewhere (McLachlan et al., 1979; Brown and McLachlan, 1990). McLachlan et al. (1979) suggested that occurrence in the swash zone is related to feeding. Generally, *Ovalipes* species are active nocturnally (Caine, 1974; Du Preez, 1983). Our observations of foregut fullness and abundance support these findings. Furthermore, because peracarid crustaceans that live close to the sandy shoreline dominated the foregut contents (Kamihira, 1979; Takahashi and Kawaguchi, 1995; authors' pers. obs.), we concluded that part of the crab population migrates from deeper waters into the swash zone at night to exploit the crustacean fauna of sandy beaches.

Feeding in the swash zone is advantageous for the crabs because important prey, such as the amphipod *H. japonicus*, the mysid *A. kokuboi*, and the isopod *E. chiltoni*, are abundant in the swash zone of Koshirahama Beach. These species are considerably more abundant near the shoreline than other macrobenthic organisms that live in the offshore area (authors' pers. obs.). The peracarids, especially mysids and isopods, burrow into the sand during daytime, then emerge into water column in the swash zone at night (Takahashi and Kawaguchi, 1997; authors' pers. obs.) which makes them more vulnerable to predation by *O. punctatus* and fishes (Takahashi et al., 1999). The predominance of peracarid crustaceans in the foreguts of the crabs suggests that *O. punctatus* capture the peracarids effectively in the swash zone of Koshirahama Beach at night.

Species of *Ovalipes* are known as opportunistic, broad-spectrum predators. They are extremely versatile, feeding on both less active prey, such as mollusks, and on mobile animals, such as amphipods, mysids, isopods, and fish (Caine, 1974; Haefner, 1985; Wear and Haddon, 1987; Ropes, 1989; Stehlik, 1993).

The swash zone also serves as a refuge from predation. Species of *Ovalipes* (including congeneric species) are occasionally found in the guts of fish and decapod crustaceans (McDermott, 1983; Du Preez and McLachlan, 1984; Mitchell, 1984; Wear and Haddon, 1987; Stehlik, 1993). Cannibalism is a major cause of mortality in many portunid crabs during their early life stages; the importance of refuges has been emphasized as a factor regulating crab recruitment in natural populations (Hines and Ruiz, 1995). In estuarine ecosystems, habitats with structural complexity, i.e. seagrass beds, mussel beds, filamentous algae, are effective as refuges for juvenile crabs, whereas risk of predation is higher in open sand, the main habitat of *Ovalipes* (Wilson et al., 1987; Ryer et al., 1997, Moksnes et al., 1998). In sand habitat, small *Ovalipes* burrow deep by using reverse gill current to hide from predators during the day (Barshaw and Able, 1990).

Nocturnal emergence of *Ovalipes* makes them more vulnerable to cannibalism. In the swash zone of Koshirahama Beach at night, almost all the *O. punctatus* were juvenile and immature crabs. This finding suggests that small individuals avoid the deeper zone, where the risk of cannibalism is high. For blue crabs, *Callinectes sapidus*, shallow water areas function as refuges from predation

when habitats have little structural complexity (Dittel et al., 1995; Hines and Ruiz, 1995). The swash zone is probably difficult for larger predators to penetrate and they risk being stranded. In addition, birds that exploit the swash zone are less active at night (Brown and McLachlan, 1990).

Softshell and premolt crabs were not collected in the swash zone; it appears that this zone may not be a molting ground owing to its dynamic characteristics. However, most diurnal and adult crabs in the samples were papershell crabs, which are also vulnerable to predation; the swash zone, therefore, may be important as a refuge for postmolt crabs.

In our study, juvenile and immature crabs dominated the population of *O. punctatus* in the swash zone during late summer. From the size of the crabs, these were first-year crabs born in the previous winter or spring, and therefore their occurrence in the swash zone may be limited to the summer and fall, when these juvenile and immature crabs appear (Sasaki and Kawasaki, 1980). A similar seasonal occurrence of juvenile *O. ocellatus* from July to October on exposed sandy beaches of the mid-Atlantic Coast of the United States has been reported in an inshore area (McDermott, 1983). The occurrence and behavior of *O. punctatus* in the swash zone suggest that this habitat is important as a feeding ground and a refuge from predation. Intertidal sand-burrowing peracarids, the major diet items of *O. punctatus*, also consume particulate organic matter and organisms in the swash zone (Kamihira, 1992; Takahashi and Kawaguchi, 1998). Thus by feeding in the swash zone, young *O. punctatus* transport some of the secondary production from the swash zone to the offshore area through their ontogenetic migration into deeper water.

Acknowledgments

We are grateful to Captain K. Morita and T. Kawamura, and K. Hirano of the Otsuchi Marine Research Center, Ocean Research Institute (ORI), for their help in field sampling. We would like to thank C. Vallet of Soka University who translated the French articles. We also thank T. P. Hirose of the Japan Sea National Fisheries Research Institute and the staff of the Plankton Laboratory of ORI for their help with fieldwork and for fruitful discussions during the course of our study.

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Errata

***Fishery Bulletin* 98(4):759–766**

Lindley, Steven T., Michael S. Mohr, and Michael H. Prager

Monitoring protocol for Sacramento River winter chinook salmon, *Oncorhynchus tshawytscha*: application of statistical analysis to recovery of an endangered species

Erratum 1: page 759, right column, line 10. The equation within the text should read

$$R_t = N_t / N_{t-3}.$$

Erratum 2: page 760. Equation 1 should read

$$t = \frac{\bar{r} - \rho_{goal}}{s / \sqrt{n}}.$$

Erratum 3: page 761. Equation 2 should read

$$\delta = \frac{\rho - \rho_{goal}}{\sigma / \sqrt{n}}.$$

***Fishery Bulletin* 99(1):49–62**

Gharrett, Anthony J., Andrew K. Gray, and Jonathan Heifetz

Identification of rockfish (*Sebastes* spp.) by restriction site analysis of the mitochondrial ND-3/ND-4 and 12S/16S rRNA gene regions

Erratum: page 56, line 5

Read "*S. melanops*" instead of "*S. maliger*" so that the sentence begins

"Similarly, the haplotypes of *S. melanops* and *S. flavidus* (subgenus *Sebastosomus*) were tightly clustered…".

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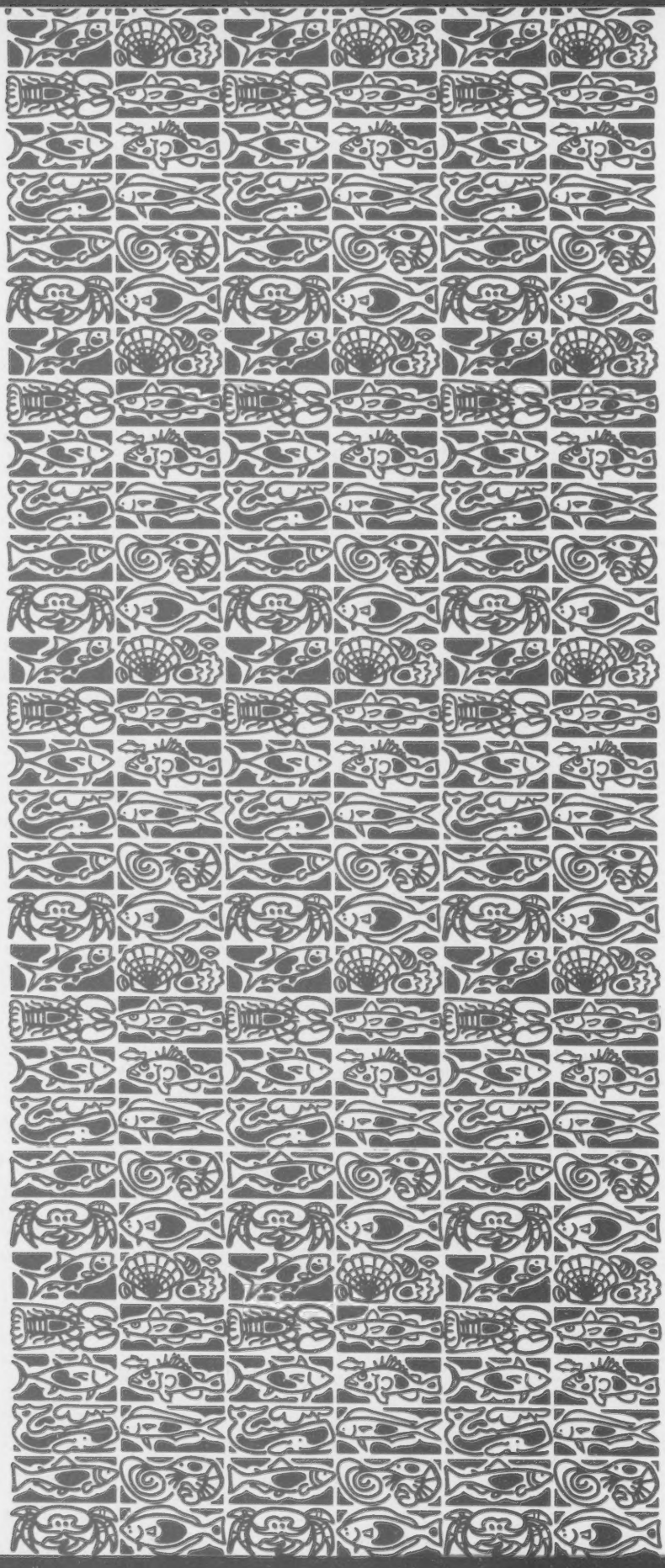
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